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YOUNG INVESTIGATOR PERSPECTIVE

Melanocortin Signalling and the Regulation of Blood Pressure in Human Obesity

J. R. Greenfield

Garvan Institute of Medical Research, St. Vincent's Hospital and University of New South Wales, Sydney, NSW, Australia.

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Obesity has reached epidemic proportions worldwide. Sympathetic nervous system activation has been shown to play a major role linking obesity to the development of associated metabolic complications, such as hypertension. Recent evidence has implicated central melanocortin signalling in the regulation of blood pressure in rodents and humans. The importance of sympathetic neural activity in mediating this association has been highlighted. Humans with loss-of-function mutations in the melanocortin 4 receptor (MC4R) are an ideal group of subjects in whom the importance of melanocortin signalling in linking obesity to hypertension can be studied. Consistent with rodent studies, it was recently demonstrated that humans with MC4R deficiency have lower blood pressure, less hypertension, lower 24-h urinary catecholamine excretion, lower resting heart rate and attenuated insulin-mediated sympathetic activation compared to equally-obese humans. In overweight and obese humans without MC4R mutations, the infusion of a highly-selective MC4R agonist led to dose-dependent increases in blood pressure and heart rate. All effects were independent of insulin. This evidence supports the notion that the melanocortin system regulates blood pressure and sympathetic neural function. The results obtained in rodent and human studies, in relation to blood pressure and sympathetic function, may limit the use of MC4R agonists for the treatment of obesity. Future studies will determine whether MC4R deficiency is associated with protection from development of the detrimental cardiovascular consequences that accompany obesity.

Key words: Obesity, blood pressure, melanocortin 4 receptor, sympathetic nervous system.

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Correspondence to:
Dr J. R. Greenfield, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst NSW 2010, Australia (e-mail: j.greenfield@garvan.org.au).

Obesity has reached epidemic proportions in developed and developing countries and is a major health problem affecting morbidity and mortality (1). The pathogenesis of obesity and the mechanisms that contribute to the accumulation of excess body fat are the focus of many research groups worldwide.

Obesity is associated with a number of metabolic abnormalities, including hypertension. Data from animal studies suggest that the association between adiposity and blood pressure is tightly regulated, with experimental weight gain leading to an increase in plasma volume and blood pressure (2) and weight loss resulting in reciprocal blood pressure changes (3), although not universally (4). The concept of a tight link between adiposity and blood pressure was challenged over 10 years ago, as elegantly outlined in a review of the role of leptin in regulation of sympathetic activity and blood pressure in mouse models of obesity (5). This review questioned the prevailing belief that obesity was consistently associated with hypertension and suggested that the effect of increased adiposity on blood pressure may be critically modulated by genetic–neurobiological mechanisms underlying obesity, such as reduced leptin and melanocortin signalling (5, 6).

The mechanisms that explain the link between adiposity and blood pressure have been assumed to be dependent on insulin resistance and insulin-induced stimulation of sympathetic nervous system activity, although most of the studies on which these conclusions are based are cross-sectional or short-term interventions (3, 7, 8). The dogma that hyperinsulinaemia is the mechanism of obesity-associated hypertension has been challenged. Indeed, insulin has been shown to be an important vasodilator, which would be expected to decrease (rather than increase) blood pressure and related measures, such as arterial stiffness (9). Studies in dogs and humans (10, 11) have demonstrated that, despite insulin-induced sympathetic stimulation, a powerful direct vasodilator action of insulin decreases vascular resistance and does not increase arterial pressure. Other postulated mediators of the obesity-hyperinsulina-

emia-hypertension link include excess renal sodium retention and alterations in ion transport (12, 13).

The leptin–melanocortin pathway is an important neuronal circuitry that controls food intake and body weight (14). In both rodents and man, disruption of this pathway results in hyperphagia and severe obesity (15). Recent evidence in rodents and humans has also implicated central melanocortin signalling in the regulation of sympathetic neural activity and blood pressure control (16, 17), suggesting that the leptin–melanocortin pathway may play an important role in mediating weight gain-associated hypertension, via increased sympathetic activity.

The present review discusses the links between obesity and sympathetic nervous system function and outlines the evidence in favour of central leptin–melanocortin signalling in the regulation of sympathetic activity and blood pressure control.

Obesity, energy balance and sympathetic nervous system activity

Energy homeostasis is a tightly controlled system integrating energy intake and energy expenditure. The sympathetic nervous system is considered to be an important player that controls both metabolic processes. Indeed, increasing sympathetic activity induces satiety and leads to a reduction in food intake (18, 19). Sympathetic nervous system activation using β-3 adrenergic agonists decreased food intake in lean and obese Zucker rats, an effect abrogated by previous treatment with a β-adrenergic antagonist (20). In relation to energy expenditure, acute sympathetic activation has been shown to lead to increased metabolic rate, increased diet-induced thermogenesis, enhanced fat oxidation, increased lipolysis in adipose tissue and reduced adipocyte proliferation (21–24).

Obesity is associated with increased sympathetic activity (25–27), most likely driven by hyperinsulinaemia and insulin resistance (28). Leptin, an adipokine, has also been shown to activate central sympathetic outflow within the hypothalamus, although an effect on peripheral neural activity cannot be excluded. Previous studies demonstrate that the action of leptin in the arcuate nucleus of the hypothalamus controls sympathetic outflow to both brown adipose tissue and the kidney (29). Coupled with hyperinsulinaemia, leptin may be a potential mechanism by which the sympathetic nervous system is activated in obesity (30), perhaps as an adaptive response, in an attempt to maintain body weight homeostasis (31).

There is evidence that obesity is characterised by selective resistance to leptin- and insulin-mediated sympathetic nervous system activation, with resistance to the metabolic effects of leptin, but preservation of sympathetic activation to the kidney, leading to hypertension. In a study of high fat-fed obese mice, lumbar (skeletal muscle) and brown adipose tissue sympathetic activity was attenuated in response to intraperitoneal or intracerebroventricular leptin, whereas the renal sympathetic response to leptin was preserved (32). In humans, it has been shown that, despite sympathetic nervous system activation, responsiveness to insulin is attenuated in obesity (33–35). In the postprandial state, this would be expected to lead to impaired diet-induced thermogenesis and post-prandial fat oxidation, promoting the accumulation of body fat.

Central leptin–melanocortin signalling

The central leptin–melanocortin pathway has been shown to be an important regulator of food intake and body weight in both nonhuman animals and humans. In the mammalian hypothalamus, melanocortin neurones orchestrate an interconnected neuroendocrine and autonomic circuitry, maintaining energy homeostasis through the monitoring of caloric intake, energy expenditure and the regulation of behavioural and metabolic responses to nutritional fluctuations (36). Activation of hypothalamic leptin receptors in the arcuate nucleus results in proteolytic cleavage of pro-opiomelanocortin (POMC) by prohormone convertases, yielding the anorectic peptide α-melanocyte-stimulating hormone (α-MSH). α-MSH is a natural ligand for the melanocortin 4 receptor (MC4R), a 332 amino acid seven-transmembrane G protein-coupled receptor, abundantly expressed in the hypothalamic paraventricular and other nuclei (37). As outlined below, disruption of key nodes in this pathway manifest as severe hyperphagic obesity in rodents and humans.

Central melanocortin signalling in the regulation of sympathetic neural activity and blood pressure in nonhuman animals

Recent studies in nonhuman animals suggest that leptin–melanocortin signalling plays a key role in regulating cardiovascular function via alterations in sympathetic nervous system activity. In rodents, leptin effects on appetite and energy expenditure are associated with dose-dependent increases in sympathetic nerve activity in brown adipose tissue and the kidneys (38). These effects are abrogated in rats without functional leptin receptors (38). In a recent study of obese and normal-weight rabbits, i.c.v. leptin increased arterial pressure and renal sympathetic traffic, particularly in obese animals (39). Leptin has also been shown to restore cardiac sympathetic–vagal balance and baroreflex sensitivity in diabetic rats (40). The sympathoexcitatory effects of leptin appear to be dependent on activation of melanocortin neurones because they are significantly attenuated in mice with reduced or absent MC4R (41). Intracerebroventricular administration of an MC4-R agonist resulted in dose-dependent sympathoexcitation of brown adipose tissue and renal and lumbar beds, a response completely blocked by the MC4-R antagonist SHU-9119 (42).

MC4R-deficient mice are obese, as a result of an inability to co-ordinate energy intake and energy expenditure; their phenotype includes hyperphagia, reduced resting energy expenditure and defective diet-induced thermogenesis (43, 44). In addition, homozygous and heterozygous MC4R-deficient mice are protected from developing hypertension, despite becoming obese (45). Interestingly, MC4R-deficient mice have lower heart rates than their wild-type littermates. In another study (16), acute central administration of α-MSH increased mean arterial pressure and heart rate in wild-type animals but not in MC4R knockout mice, suggesting a role for central melanocortin receptors in mediating the cardiovascular effects of α-MSH. Furthermore, pretreatment with an alpha adrenergic receptor blocker (phentolamine) abrogated the effect of α-MSH on arterial pressure and heart rate, providing evidence that

the cardiovascular effects of α-MSH are attributable to sympathetic nervous system activation.

Despite increased food intake and significant weight gain, chronic antagonism of MC3/4R by intracerebroventricular infusion of SHU-9119 resulted in lower heart rate and mean arterial pressure, particularly in the spontaneously hypertensive rat, a model of increased sympathetic activity (46). These effects, particularly the decrease in blood pressure, were shown to be a result of the inhibition of adrenergic activity. Other studies also confirm a role of the MC4R in modulation of sympathetic neural activity and cardiovascular regulation (47–49).

From an anatomical perspective, it has been shown that MC4R-expressing neurones are present in hypothalamic paraventricular nuclei, allowing access to autonomic preganglionic neurones (50, 51). Taken together, the results of nonhuman animal studies suggest that functional melanocortin signalling is important for obesity-associated sympathetic overdrive and the development of hypertension, and that interruption of this pathway will result in dissociation of obesity from hypertension.

Monogenic obesity disorders in humans

Using a candidate gene approach, mutations in a number of key components of the leptin–melanocortin pathway, including *leptin*, *leptin receptor*, *POMC*, *prohormone convertase 1* and *MC4R*, have been described in humans with hyperphagia and severe early-onset obesity (14, 52, 53). By far the commonest form of human monogenic obesity, albeit still relatively rare, is a result of mutations in *MC4R*, with a prevalence of 6% in severely obese subjects with onset of obesity in childhood (52), 2.5% in a study of 750 obese Danish males with a history of juvenile-onset obesity (54) and 0.1% in a population-based study of unselected individuals living in England (55). The key clinical characteristics of human MC4R deficiency, similar to those observed in the mouse, are early-onset hyperphagic obesity, increased lean body mass, accelerated linear growth, increased final adult height, increased bone mineral density and disproportionate fasting hyperinsulinaemia (52, 56). As discussed below, MC4R deficiency is also characterised by protection from the development of hypertension.

MC4R deficiency is inherited in a co-dominant fashion, with heterozygotes exhibiting a less severe phenotype than the comparatively small number of homozygotes that have been described to date (52). Furthermore, there is a strong correlation between the *in vitro* functional properties of mutant MC4Rs and many phenotypic features of MC4R deficiency, such as hyperphagia, obesity and hyperinsulinaemia (52). Interestingly, the clinical phenotype is most prominent in childhood, with the difference between affected and unaffected obese individuals becoming less marked with age (52).

The autonomic nervous system and its measurement in humans

The autonomic nervous system is a complex system of nerves and ganglia involved in the control of involuntary activity, comprising two arms: the sympathetic and the parasympathetic nervous system. Various techniques have been used to measure autonomic nervous system activity in humans (57).

Whole-body sympathetic activity is most commonly assessed by measuring plasma, platelet or 24-h urinary catecholamines and their metabolites. Other measures used are noradrenaline turnover (spillover and clearance) following infusion of radioactive noradrenaline and microneurography to measure muscle sympathetic nerve activity (MSNA), a direct indicator of central sympathetic outflow to the vasculature.

Autonomic nervous system function can also be assessed by spectral analysis of heart rate variability, which provides specific information regarding both sympathetic and parasympathetic (vagal) nervous system function. Various cardiac autonomic function measures are derived from heart rate variability.

Melanocortin signalling plays a role in the regulation of sympathetic neural activity and blood pressure regulation in humans

Humans with MC4R mutations are a unique group in whom the linkage of melanocortin signalling in obesity to hypertension can be studied. In a recent study, evidence was provided for a role of neuronal melanocortins in the modulation of blood pressure in humans (17). Blood pressure, heart rate, urinary catecholamines and heart rate variability were examined in overweight and obese humans with loss-of-function mutations in *MC4R* and equally overweight and obese controls. The first finding was that the prevalence of hypertension in the subjects with MC4R deficiency was approximately half that compared to controls (Fig. 1A). Second, systolic and diastolic blood pressure was lower in MC4R-deficient humans, compared to controls, after the exclusion of subjects taking antihypertensive medications (Fig. 1B,C). Third, a subgroup of ten adults with haploinsufficiency for MC4R had lower 24-h urinary norepinephrine excretion compared to controls (Fig. 2). Fourth, despite similar sleeping heart rates in eight MC4R-deficient adults of European decent and eight controls, matched for age, ancestral background and body mass index (BMI), MC4R subjects exhibited an attenuated heart rate response to waking (Fig. 3A). The difference in heart rate between the groups persisted during the hyperinsulinaemic–euglycaemic clamp, with a mean difference of seven beats per minute during the steady-state period. Measures of parasympathetic function exhibited the opposite effect (Fig. 3B,C). By contrast to control subjects, who experienced progressive reduction in measures of parasympathetic function (high frequency component and the root mean square of successive differences between adjacent normal RR intervals) after waking and during the hyperinsulinaemic–euglycaemic clamp, MC4R-deficient subjects experienced no reduction in either of these measures upon waking, with only a small reduction during the hyperinsulinaemic–euglycaemic clamp, such that parasympathetic measures remain significantly higher in MC4R-deficient subjects compared to controls throughout the clamp period. Interestingly, the low frequency component heart rate variability, a composite measure of sympathetic and parasympathetic function, did not differ between the two groups at any time point.

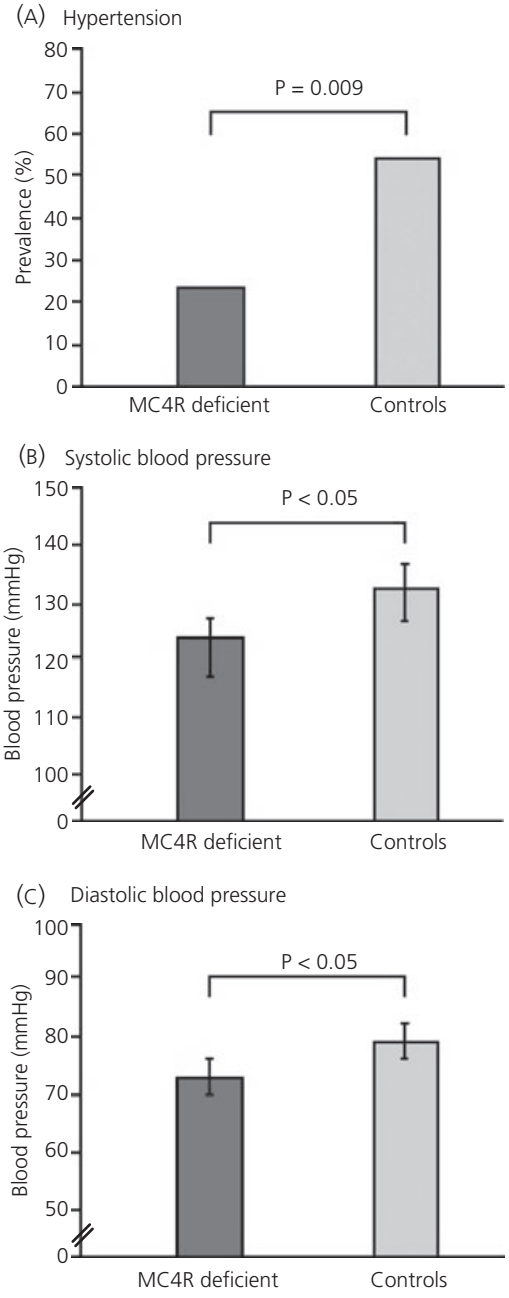

Fig. 1. Prevalence of hypertension (A) and blood pressure measures (B, C) in 46 subjects with melanocortin 4 receptor (MC4R) deficiency, as compared with 30 overweight or obese control subjects. Four subjects with MC4R deficiency and seven control subjects were excluded from the analyses in (B) and (C) because they were taking antihypertensive medications. Vertical bars represent the standard error. Reproduced from the New England Journal of Medicine with permission.

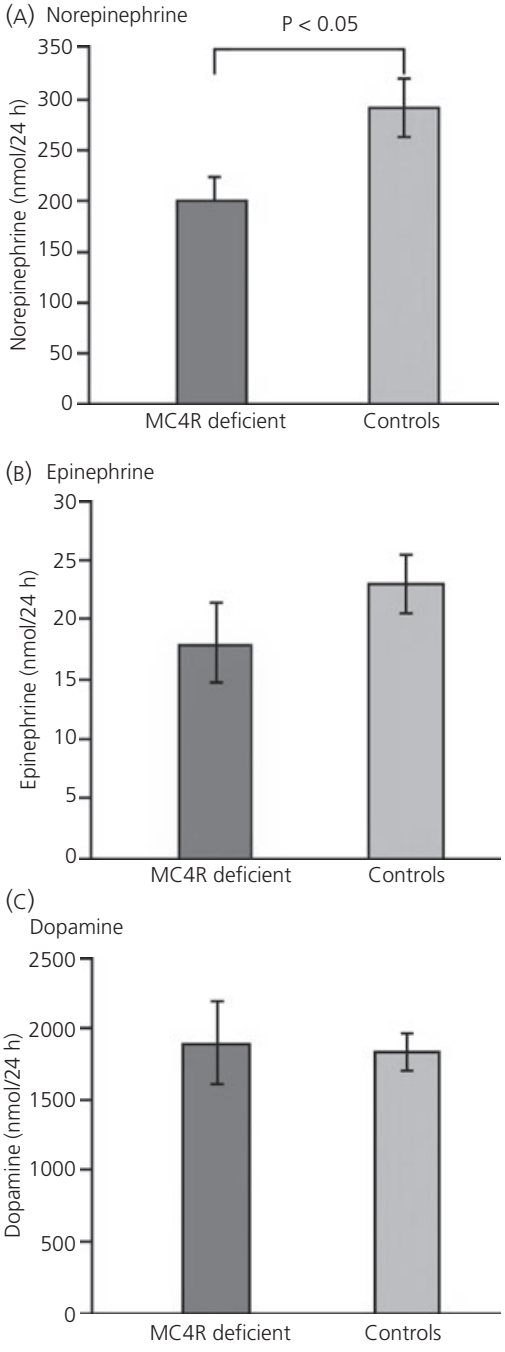

Fig. 2. Twenty-four-hour levels of urinary norepinephrine (A), epinephrine (B) and dopamine (C) in ten subjects with melanocortin 4 receptor (MC4R) deficiency and 19 control subjects. Vertical bars represent the standard error. Reproduced from the *New England Journal of Medicine* with permission.

In a separate cohort of 28 healthy overweight or obese subjects, subcutaneous infusion of a highly-selective MC4R agonist (LY2112688) led to a dose-dependent increase in blood pressure over a 24-h period. Indeed, 3 h after the infusion, systolic blood pressure was 4.0–8.5 mmHg higher and diastolic blood pressure was 2.5–8.2 mmHg higher than placebo-control values (Fig. 4). The mean ± SD differences in systolic and diastolic blood pressure at 24 h were 9.3 ± 1.9 and 6.6 ± 1.1 mmHg for the maximal tolerated dose of 1.0 mg of the agonist. The average heart rate increased during the 7-day infusion, with a difference of three beats per minute compared to placebo.

As discussed above, it is widely considered that insulin resistance and hyperinsulinaemia, which accompany obesity, are pathogenic

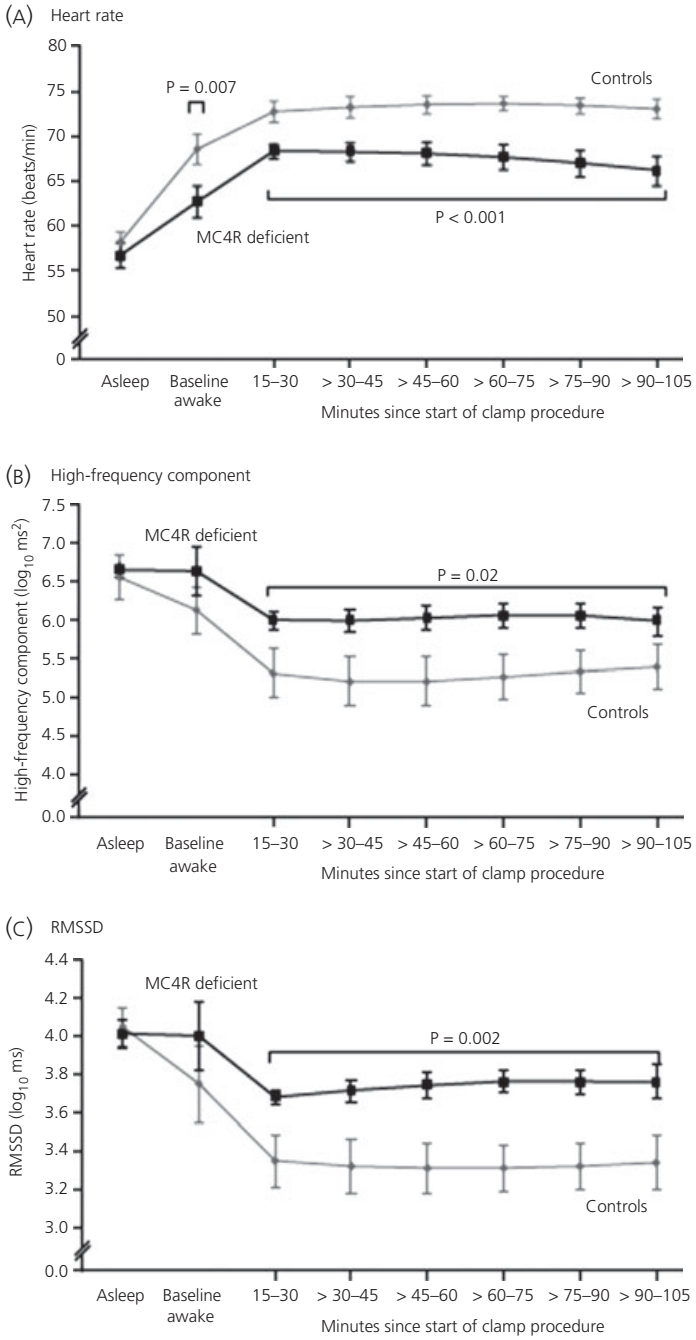

Fig. 3. Heart rate (A), its high-frequency component (B) and the root mean square of successive differences (RMSSD) between adjacent normal RR intervals (C) (latter two measures of parasympathetic activation) during sleep, in the awake state, and during the hyperinsulinaemic–euglycaemic clamp procedure in eight subjects with melanocortin 4 receptor (MC4R) deficiency and eight control subjects. Vertical bars represent the standard error. Reproduced from the New England Journal of Medicine with permission.

factors in the development of hypertension, via alterations in vascular function, renal sodium handling and sympathetic nervous system activation (8). The above findings do not support this notion. First, despite similar adiposity, insulin sensitivity and circulating insulin levels, MC4R-deficient individuals had lower blood pressure than controls. Second, infusion of the MC4R agonist LY2112688 led to dose-dependent increases in blood pressure in overweight or obese adults during a 24-h period, with no change in insulin levels.

A study that extends these findings has recently been published (58). Investigators recruited eight morbidly obese (BMI = 38 kg/m^2) subjects with mutations in MC4R (seven heterozygotes and one compound heterozygote) and eight obese controls (BMI = 33 kg/m^2). Neurophysiological assessment of sympathetic function was con-

ducted by MSNA. Diastolic blood pressure, heart rate and resting MSNA tended to be lower in subjects with MC4R mutations, although the differences between groups did not reach statistical significance. Stimulated MSNA during apnoea was lower in MC4R-deficient subjects. Amongst MC4R-deficient subjects, BMI and leptin were negatively correlated with resting MSNA. Interestingly, the most obese individual (the compound heterozygote) who had a BMI of 70.8 kg/m^2, had extremely low MSNA. By contrast, there were no significant relations between measures of adiposity and sympathetic nervous system function in the control group. Although the numbers are small, these data are consistent with our findings and support an important role for MC4R in the regulation of sympathetic nerve function and blood pressure control in humans.

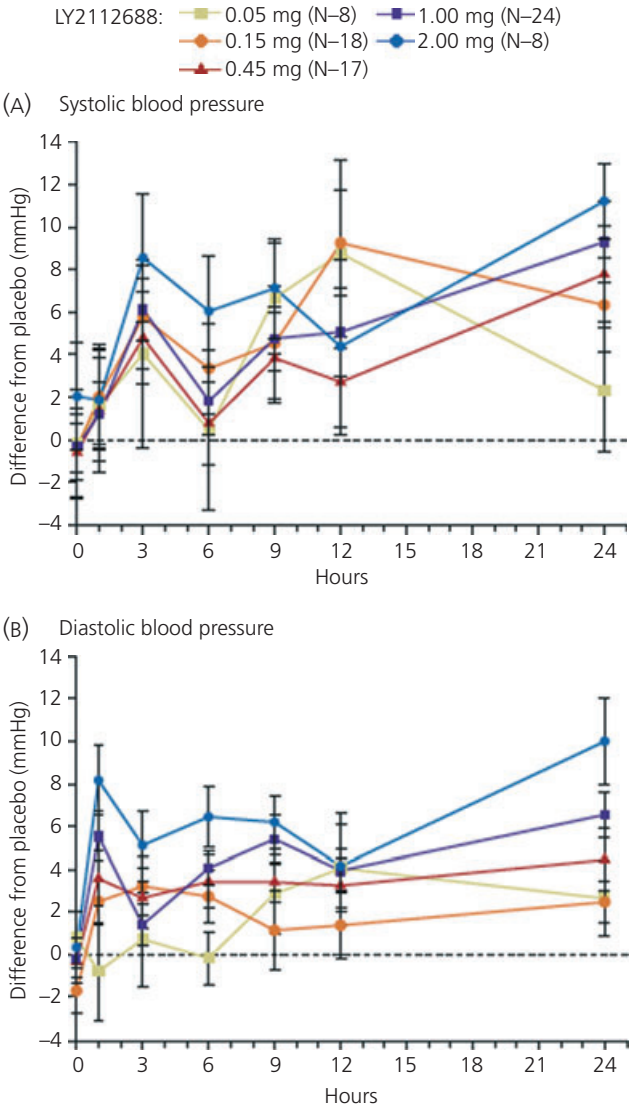

Fig. 4. Differences in mean blood pressure in subjects receiving melanocortin agonist (LY2112688) or placebo at 24 h, according to dose. The graphs show the differences from placebo in mean systolic blood pressure (A) and diastolic blood pressure (B) in 28 subjects receiving a melanocortin agonist, according to the dose of the melanocortin agonist. Vertical bars represent 90% confidence intervals. Reproduced from the New England Journal of Medicine with permission.

Contribution of reduced sympathetic nervous system activity to the obese phenotype of MC4R deficiency

In human MC4R deficiency, the contribution of defective energy expenditure (and the specific role of each metabolically-active tissue) to the obese phenotype is unclear. In non-human animals, brown adipose tissue, which is sympathetically-innervated, is known to contribute to resting energy expenditure and diet-induced thermogenesis. Contrary to conventional belief of the absence of brown adipose tissue in humans, recent advances in positron emission tomography-computed tomography, using flurodeoxyglucose as a glucose analogue, reveal substantial brown adipose tissue depots in a significant proportion of adult humans (59, 60). The presence of brown adipose tissue is associated with lower body weight and fasting glucose levels (59, 60), suggesting regulatory links between brown adipose tissue activity and energy homeostasis in humans.

In MC4R-deficient mice, thermogenic failure has been shown to stem from defective brown adipose tissue recruitment and failure to up-regulate uncoupling protein-1 when challenged by a high fat diet (61). Other evidence implicates MC4R as playing an important role in the sympathetic control of lipid mobilisation from white adipose tissue (62). In light of these results, and the recently recognised regulatory role of brown adipose tissue in energy balance in adult humans, MC4R signalling may play a critical role in regulating white and particularly brown adipose tissue activity in humans, although the quantitative contribution of brown adipose tissue relative to muscle thermogenesis needs to be determined.

Conclusions and summary

This review has summarised the evidence linking central melanocortin signalling to the regulation of blood pressure. The recognition that the melanocortin system is a regulator of blood pressure and sympathetic neural function raises the possibility that this pathway comprises a potential novel target for the modulation of blood pressure in humans. The lower blood pressure and heart rate in humans with MC4R deficiency, and the reciprocal effects of MC4R agonism on these parameters, may limit the applicability of MC4R agonists for the treatment of obesity (63). Future studies are required to determine whether humans with MC4R deficiency are protected from the development of the detrimental cardiovascular consequences that accompany obesity over the longer term.

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