Role of the Autonomic Nervous System and Neuropeptides in the Development of Obesity in Humans: Targets for Therapy?

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Abstract: Obesity and type 2 diabetes have reached epidemic proportions worldwide. These metabolic disorders, particularly obesity, are characterised by increased basal sympathetic nervous system (SNS) activity but an impaired sympathetic response to certain stimuli, such as insulin. Although targeting the SNS may seem an attractive avenue for the pharmacological prevention and treatment of obesity and related metabolic disorders, it remains unknown whether changes in SNS tone are primary and contribute to the development of these metabolic conditions or whether they develop secondary to the obese state. This question can be answered by the study of insulin-resistant individuals prior to the development of obesity and type 2 diabetes. Using this model, it has been shown that early insulin resistance is associated with increased SNS activity in genetically-predisposed humans. It has been suggested that in insulin-resistant states, hyperinsulinaemia is the initiating factor that increases sympathetic neural activity. Over time, adrenoreceptor down-regulation and/or reduced sensitivity are likely to develop, resulting in reduced sympathetic responsiveness. In the postprandial state, this will lead to impaired diet-induced thermogenesis and post-prandial fat oxidation, promoting the accumulation of body fat. More recent evidence demonstrates that stress-induced SNS overactivity up-regulates Neuropeptide Y, an orexigenic hormone, and its Y2 receptor, in visceral adipose tissue, the fat depot most strongly linked to insulin resistance and type 2 diabetes. There is evidence that SNS overactivity specifically contributes to the development of abdominal obesity via this pathway, which could represent a novel target for the prevention and treatment of abdominal obesity and related metabolic consequences.

Key Words: Autonomic nervous system, sympathetic nervous system, insulin resistance, overweight, obesity, neuropeptide Y, Y2 receptor.

ADVERSE METABOLIC EFFECTS OF OBESITY

Obesity and type 2 diabetes have reached epidemic proportions. Obesity, specifically central abdominal obesity, is a strong determinant of insulin resistance, defined as an attenuated biological response to insulin. Insulin resistance and central obesity are core components of the 'Metabolic Syndrome', which also includes dyslipidaemia, glucose intolerance and hypertension [1]. This cluster of metabolic abnormalities not only predicts type 2 diabetes mellitus, but is also associated with a significantly increased risk of cardiovascular disease, possibly in excess of that from traditional risk factors [1].

Excess fatty acids released from adipose tissue accumulate in insulin-sensitive tissues, such as muscle and liver, leading to an impairment of insulin-mediated glucose uptake ('lipid supply hypothesis of insulin resistance') [2]. Much research has been undertaken to identify specific defects in the insulin-signaling cascade that impair insulin-mediated glucose uptake, including the mechanisms by which lipid oversupply to insulin-sensitive tissues may lead to insulin resistance [3]. In contrast to peripheral adipose tissue accumulation, which appears not to be associated with adverse metabolic consequences and may even be protective, central abdominal fat is more labile, with lipolysis being less suppressed by insulin and most fatty acids draining directly into the portal system [1].

As well as acting as a source of fatty acids, adipose tissue produces hormones that modulate insulin action. It has been shown that adipocytokines, or peptide messengers, including adiponectin, resistin, tumour necrosis factor (TNF)-α and leptin, are secreted by adipose tissue, negating the belief that adipose tissue is a passive reservoir for triglycerides and fatty acids [3]. There is evidence suggesting that TNF-α and resistin may impair insulin action *in vitro*, although the exact role and source of cytokines in man remain controversial [2].

It has been suggested that alterations in autonomic nervous system (ANS) activity and function may play an role in the development of the metabolic disorders that define the Metabolic Syndrome, particularly obesity and type 2 diabetes [4]. However, it remains unclear whether defects in ANS tone are a primary aetiological factor contributing to obesity and type 2 diabetes or, rather, whether changes in ANS activity are a consequence of the obese state.

MEASUREMENT OF ANS FUNCTION

The ANS is a complex system of nerves and ganglia primarily involved in the control of involuntary activity. It comprises two pathways, the sympathetic nervous system (SNS) and the parasympathetic nervous system. As discussed in detail elsewhere, a number of techniques can be used to measure ANS activity in humans [4-7]. Whole-body sympathetic activity can be ascertained by the measurement of catecholamines (and their metabolites) in plasma, platelets or urine. More recently, techniques such as isotope dilution-derived measurements of noradrenaline release to plasma, and microneurography, which measures muscle sympathetic nerve activity (MSNA), have been utilised [5, 6]. ANS function can also be assessed by spectral analysis of heart rate variability (HRV), which provides information regarding both sympathetic and parasympathetic (vagal) nerve activity [7].

Spectral analysis of HRV has also been combined with 'stimulatory testing', in order to detect differences between groups that may not be apparent in the basal or unstimulated state. As discussed elsewhere [8], insulin has unequivocally been shown to stimulate the SNS in man. One method by which insulin can be delivered in a research setting is via the hyperinsulinaemic-euglycaemic clamp, the 'gold-standard' research tool used to measure insulin sensitivity. A major advantage of using the clamp for this purpose is that it allows one to examine the effect of hyperinsulinaemia *per se* on these parameters, in the absence of changes in glycaemia (as the glucose is clamped at 5 mmol/L).

PHARMACOLOGICAL OBESITY TREATMENT – CURRENT THERAPIES

In general, most anti-obesity medications exert their effects centrally, by inhibiting food intake and increasing energy expenditure, or peripherally, by reducing the absorption of fat in the gut. Sibutramine, a tertiary amine that enhances satiety (by blocking

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reuptake of the neurotransmitters serotonin and noradrenaline) and increases thermogenesis (by enhancing peripheral noradrenaline function), leads to modest weight loss. Sibutramine has been associated with a rise in systolic and diastolic blood pressure and a small increase in heart rate [9], mandating regular monitoring of these cardiovascular parameters. Its safety in patients with known ischaemic heart disease is under evaluation [10]. Orlistat, a reversible inhibitor of intestinal lipase, inhibits dietary fat absorption by approximately 30%, also leading to moderate weight loss [11]. A new class of anti-obesity medications, the cannabinoid (CB)-1 receptor antagonists (such as rimonabant), has recently been approved for use in Europe, although its approval in the US has been delayed due to concerns regarding depression. Sympathomimetic agents, such as phentermine, are indicated for limited short-term use only, due to their side-effect profiles, which include pulmonary hypertension and valvular heart disease [12]. β-3 adrenoceptor agonists, which would be expected to be effective weight-loss agents due to their ability to stimulate uncoupling protein 1 and thermogenesis, have not proven efficacious in weight-loss studies in humans [12].

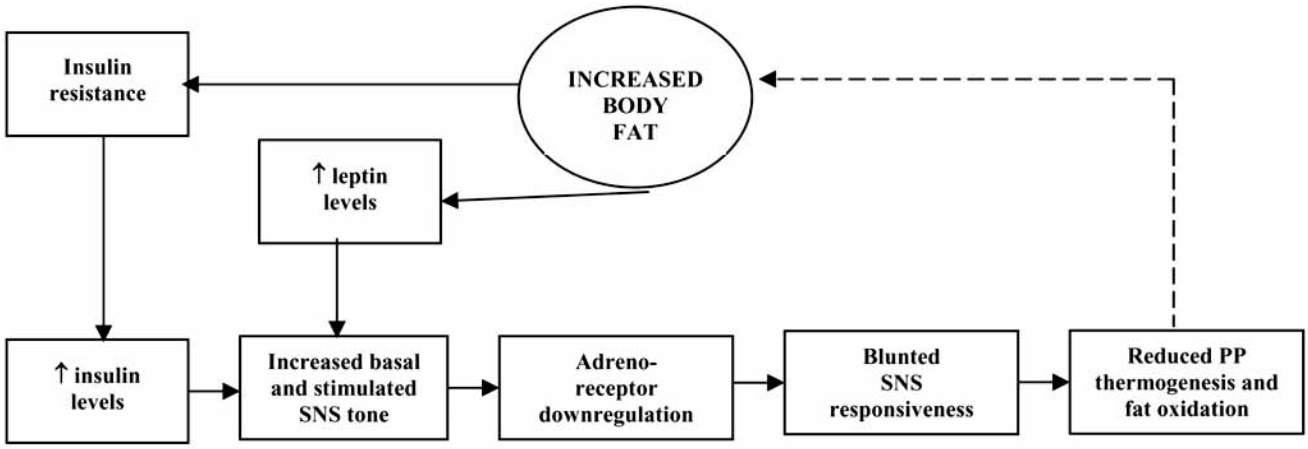
IS OBESITY CHARACTERISED BY ALTERED SNS ACTIVITY?

Whether alterations in sympathetic neural activity precede and contribute to the development of obesity in insulin resistance remains unclear. This is an important issue to resolve, as targeting the SNS may be an attractive and important avenue for the pharmacological prevention and treatment of obesity and related metabolic disorders.

In contrast to previous suggestions [13], whether alterations in ANS function are a cause or consequence of obesity cannot be ascertained from cross-sectional studies. Indeed, it has been controversial whether 'obesity' is associated with increased, decreased or unaltered sympathetic neural activity [4]. Some of the discrepancy between studies may be due to differences in the methods used to assess ANS function. Failure to measure and account for the known effects of central adiposity, insulin resistance, elevated fatty acid levels and glucose intolerance on ANS tone may also explain some inconsistency. Perhaps surprisingly, more recent studies demonstrate that a state of basal/resting sympathetic overdrive characterises obese individuals [5, 6, 13], which may be a consequence of obesity-induced hyperinsulinaemia [8]. This is biologically plausible, considering that insulin has been shown to stimulate the SNS in humans [8]. Leptin, which is secreted from adipose tissue in proportion to the degree of adiposity, has also been shown to activate central sympathetic outflow within the hypothalamus [5]. As shown in Fig. (**1**), coupled with hyperinsulinaemia, leptin may be a potential mechanism by which the SNS is activated in the obese state [14], perhaps as an adaptive response, involved in maintaining body weight homeostasis [15].

Acute activation of the SNS would be expected to induce weight loss via various mechanisms. For example, there is convincing evidence of a strong relationship between SNS activity and satiety. As reviewed in detail elsewhere by Bray and colleagues [16, 17], evidence from physiological and pharmacological studies demonstrates that increasing sympathetic activity in animals and humans leads to a reduction in food intake. For example, pharmacological activation of the SNS using β-3 adrenergic agonists decreased food intake when given intraperitoneally to lean and obese Zucker rats, an effect that was attenuated by prior treatment with a non-specific β-adrenergic antagonist [18]. Other effects of acute sympathetic activation that would be expected to contribute to weight loss include increased metabolic rate and diet-induced thermogenesis, increased lipolysis and reduced adipocyte proliferation [19-22]. Some of these mechanisms can also be inferred from studies of beta-adrenergic blockade in humans [22-24].

By definition, obesity is characterised by resistance to leptin- and insulin-mediated stimulation of SNS tone, ensuring that body weight homeostasis is maintained. This may develop as a consequence of adrenoreceptor down-regulation and/or reduced sensitivity from chronic sympathetic stimulation [20, 25] (Fig. **1**). In support of this suggestion, it has been shown that despite increased resting SNS tone, obesity is characterised by a blunted responsiveness to sympathetic stimuli, such as insulin (examined during a meal or during a hyperinsulinaemic-euglycaemic clamp) [4, 26]. In a study by Muscelli *et al.*, although the basal (resting) low frequency (LF)/high frequency (HF) ratio (a HRV measure of sympathovagal balance) was higher in obese *vs* lean individuals, insulin failed to increase the LF/HF ratio in obese subjects [27]. These findings are consistent with other reports using a similar protocol [28, 29]. Furthermore, a recent study examined the effect of physiological (meal-induced) hyperinsulinaemia on the LF/HF ratio in lean and obese subjects using high- and low-carbohydrate meals [30]. The main findings were: (i) SNS tone increased postprandially; (ii) the post-prandial increase in SNS tone was significantly greater following the high-carbohydrate meal compared to the low-carbohydrate meal; and (iii) the carbohydrate (insulin)-induced increase in SNS tone following the meal was blunted in obese subjects (despite higher postprandial insulin levels). However, it cannot be concluded from these reports that reduced sympathetic responsiveness contributes to the development of obesity, as altered sympathetic tone and responsiveness may be consequences of the obese state and, perhaps, its associated comorbidities, particularly insulin resistance.



PP = post-prandial. SNS = sympathetic nervous system.

Fig. (1). Alterations in SNS activity are a consequence of obesity.

IS SNS TONE ALTERED IN 'EARLY' INSULIN RESISTANCE?

To answer the question as to the role of alterations in the ANS in the aetiology of obesity and, consequently, type 2 diabetes, it is essential that ANS activity is examined prior to the onset of these disorders. Although a report in Pima Indians suggests that *reduced* baseline sympathetic activity (measured by 24-hr noradrenaline excretion) may predict weight gain [31], it is argued that the unique genetic make-up of the Pima Indians makes it difficult to extrapolate these findings to other populations [4].

In order to examine the effect of insulin resistance *per se* on ANS activity (i.e. independent of adiposity), some authors have recruited lean, healthy individuals, assigned to insulin-sensitive and 'less insulin-sensitive' groups and used insulin infusion during hyperinsulinaemic-euglycaemic clamp to detect differences between groups. For example, in a study by Bergholm *et al.*, insulin infusion increased 'sympatho-vagal tone' (measured by HRV) in insulin-sensitive subjects, but had no effect in 'less insulin-sensitive' individuals [32]. Interestingly, there was no difference between the groups in this measure at baseline (ie. in the unstimulated state). Furthermore, in the whole group, there was a positive relationship between insulin sensitivity measured during the clamp and the increase in sympatho-vagal tone following hyperinsulinaemia.

An alternative model is the study of individuals with a strong family history of these metabolic disorders (first-degree relatives), who may exhibit aberrations in ANS activity prior to the onset of overt disease. As stated above, insulin resistance is fundamental to the pathogenesis of the Metabolic Syndrome and type 2 diabetes and is a heritable trait. Type 2 diabetes is also strongly genetically determined. Consequently, first-degree relatives of these subjects demonstrate the metabolic accompaniments of insulin resistance before they develop overt diabetes and obesity. Because hyperglycaemia further impairs insulin action and insulin secretion, the study of primary metabolic defects leading to insulin resistance, central obesity and type 2 diabetes logically must be undertaken before insulin secretion begins to fail and blood glucose rises.

We have previously used this model to examine the role of 'classical inflammation' [33] and intramyocellular lipid accumulation [34] as pathogenic factors relating to insulin resistance. By demonstrating the absence of both of these conditions in the presence of insulin resistance, we were able to conclude that these factors do not precede and play a major role in the initiation of insulin resistance, but rather these abnormalities are more likely a later consequence of the insulin-resistant state and associated disorders, such as obesity and type 2 diabetes.

A recent study reported that basal (unstimulated) sympatho-vagal tone was increased in relatives of individuals with type 2 diabetes compared to controls, particularly overnight [35]. In a study by Laitinen *et al.* of non-diabetic offspring of patients with newly-diagnosed diabetes, insulin infusion during a clamp increased sympatho-vagal tone in relatives with an insulin-resistant phenotype (based on a high fasting C-peptide), but *not* in insulin-sensitive insulin-deficient relatives (based on a low fasting C-peptide) or controls (with no family history of type 2 diabetes) [36]. However, it should be noted that subjects with an insulin-resistant phenotype had a higher Body Mass Index (and, presumably, increased total and central abdominal adiposity), which may have influenced the results and therefore only limited conclusions should be drawn from this study. In another study of relatives of type 2 diabetic patients, a similar phenomenon was observed, with greater sympathetic stimulation during endogenous hyperinsulinaemia (an intravenous glucose tolerance test) in insulin-resistant relatives, which persisted throughout a subsequent hyperinsulinaemic-euglycaemic clamp [37].

In summary, in contrast to the findings described earlier in obesity, these studies suggest that non-obese insulin-resistant relatives of individuals with type 2 diabetes exhibit increased SNS activity in the basal and insulin-stimulated states. It is possible that insulin resistance-associated hyperinsulinaemia is the initiating factor that leads to chronic 'hyperactivation' of basal and stimulated SNS tone in relatives of type 2 diabetic individuals. This is in line with previous assertions that hyperinsulinaemia in type 2 diabetes plays a role in sympathetic hyperactivity and the development of cardiovascular complications in this population [38]. As mentioned above, it has been suggested that chronic stimulation of SNS tone leads to adrenoreceptor down-regulation and/or reduced sensitivity [20, 25]. In the post-prandial state, this would mean an impaired ability of insulin to stimulate diet-induced thermogenesis [8, 39, 40] and in particular, lipid oxidation, the substrate whose oxidation is most dependent on SNS activity [41]. In line with these suggestions, our group has recently shown that healthy relatives of people with a family history of type 2 diabetes have an impaired ability to increase fatty acid oxidation in response to a high-fat meal [42]. We are currently investigating whether post-prandial levels of noradrenaline and heart rate variability-derived estimates of ANS function are different in individuals with a strong family history of type 2 diabetes and obesity vs controls without a family history, to determine whether the 'defect' in fat oxidation is related to reduced sympathetic responsiveness to insulin. If found to be the case, this would be expected to contribute to the accumulation of body fat and an impaired ability to lose weight. Indeed, a blunted increase in the LF/HF ratio during a hyperinsulinaemic-euglycaemic clamp in severely obese individuals has been shown to independently predict reduced weight loss following bariatric surgery [43].

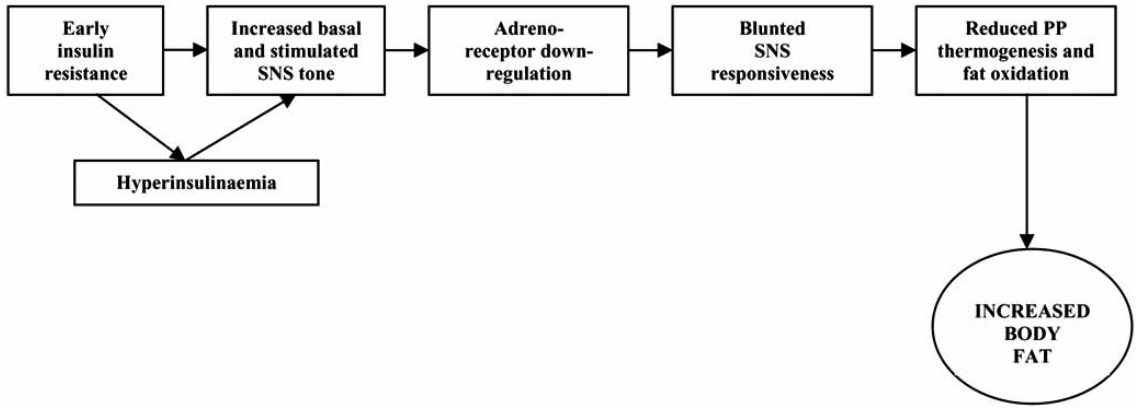
Based on the available evidence, we propose a model whereby insulin-mediated alterations in autonomic neural activity contribute to the accumulation of body fat in states of insulin resistance (Fig. **2**). Others have proposed that sympathetic activation may even contribute to the development of insulin resistance, by altering regional haemodynamics in insulin-sensitive tissues (reducing glucose transport across membranes) and/or by increasing fatty acid supply, due to enhanced rates of lipolysis [6]. However, this issue remains unresolved, with some authors providing evidence negating this suggestion [40].

From the evidence presented above, it could be surmised that insulin-resistant individuals who become obese and develop type 2 diabetes are particularly susceptible to developing beta-adrenergic down-regulation and/or desensitization in the setting of chronic adrenergic stimulation by insulin. However, it remains to be determined whether early intervention to reduce sympathetic activity will be an effective therapeutic pathway for preventing or delaying the transition from the non-obese to the obese state.

NEUROPEPTIDE Y (NPY) – THE 'MISSING LINK' BETWEEN INSULIN RESISTANCE, SYMPATHETIC NEURAL ACTIVATION AND THE ACCUMULATION OF ABDOMINAL OBESITY?

Most of the studies mentioned above have failed to account for the influence of visceral (abdominal) fat, a more important and robust determinant of metabolic disease than peripheral (subcutaneous) fat. If diseases such as type 2 diabetes are to be prevented by pharmacological targeting of the ANS, it is important to determine whether and how ANS alterations promote the accumulation of abdominal adipose tissue. It remains somewhat controversial as to the relative contribution of the sympathetic vs parasympathetic components to abdominal fat accumulation. Targeting the SNS may be important considering that sympathetic neural activation, as measured by microneurography, has been shown to be closely related to visceral adiposity (independent of subcutaneous abdominal adiposity) [44, 45].

NPY, a powerful central orexigen, is a 36-amino acid neuropeptide member of the pancreatic polypeptide family, highly expressed in and derived from the hypothalamic arcuate nucleus and sympa-



PP = post-prandial. SNS = sympathetic nervous system.

Fig. (2). Alterations in SNS activity are a cause of obesity.

thetic nerves. In rodents, chronic intracerebroventricular infusion of NPY results in obesity and muscle insulin resistance, even when NPY-induced increases in food intake are prevented [46, 47]. This suggests that there may be a direct link between central NPY release and the development of metabolic disease, at least in animals.

NPY activates a population of G-protein-coupled receptors, known as the Y-receptors, including the five cloned Y-receptors, Y1, Y2, Y4, Y5 and y6. However, it remains unclear which of these receptors are the predominant mediator(s) of NPY's effects on energy balance. Given the importance of NPY in the control of energy intake and body weight in mammals, there has been considerable interest in the therapeutic potential of specific NPY receptor subtype modulators in the management of obesity and its various metabolic accompaniments in humans. In particular, pharmacological manipulation of the Y1, Y2, Y4 and Y5 receptors has attracted significant academic and pharmaceutical interest. Despite some evidence from animal models that targeting of specific Y receptors decreases body weight, the evidence is inconsistent and not always borne out in clinical studies [48-50]. This is particularly relevant to human trials of selective Y5 receptor antagonists, which have failed to demonstrate clinically meaningful effects on body weight [51-53]. In part, this may be due to the redundancy of the peptides mediating energy homeostasis, thereby limiting the approach of targeting a single pathway in an attempt to reduce food intake and body weight. In addition, central and peripheral Y receptors can have opposing actions on parameters that affect energy homeostasis [54], so it is important to consider which receptor pool a pharmacological agent reaches. Agents that target more than one Y receptor, such as the Y2 and Y4 receptors, may prove to be more effective in inducing weight loss in clinical studies [12].

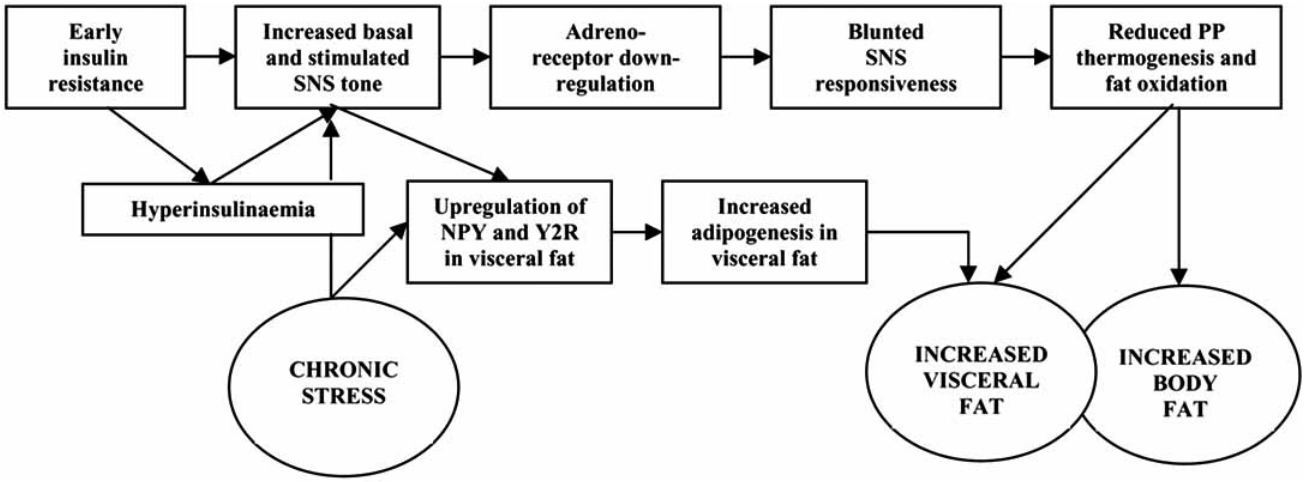
Additional evidence regarding the relative importance of individual Y receptors in mediating the obesifying effects of NPY comes from knock-out experiments in rodents [49]. Conclusions regarding the importance of these receptors in regulating body weight and adiposity are varied and conclusions from studies using germ-line knockouts of Y receptors must be limited, due to the potential effect of compensatory and secondary changes that may arise during development. In the first study to use a conditional knock-out of a Y receptor, Sainsbury *et al.* highlighted the importance of hypothalamic Y2 receptors in the regulation of adiposity in mice [55]. It has also been reported that the Y2 receptor plays a role in mediating the obese phenotype of the leptin-deficient *ob/ob* mouse [56, 57]. In humans, a common silent polymorphism of the Y2 receptor has been reported to protect against obesity [58]. Therefore, the Y2 receptor is thought to be an important mediator of the effects of NPY on energy homeostasis.

Recent evidence from animal studies suggests that NPY released from sympathetic nerves may play a key role in linking sympathetic activation to the accumulation of body fat, specifically abdominal adipose tissue. It has been proposed that in states of chronic stress, resulting in sympathetic overactivity, NPY is released from sympathetic nerves in abdominal fat tissue (in addition to and mediated by noradrenaline), leading to activation of Y2 receptors [59]. This, in turn, increases angiogenesis and adipogenesis and inhibits lipolysis, promoting abdominal adipose tissue accumulation [59], leading to insulin resistance. Stress-induced increases in adipose tissue corticosterone further up-regulate NPY and Y2 receptor expression in adipocytes [59]. It has been suggested that, over time, adipose tissue noradrenaline is depleted in states of chronic stress, lessening the weight loss-inducing effect of the β-adrenergic system, further promoting fat accumulation [59]. In rodent models, overfeeding, coupled with chronic stress, exacerbates abdominal obesity and blockade of the Y2 receptor prevents it [59]. Contrary to this evidence, Vettor *et al.* have demonstrated that unlike intracerebroventricular NPY administration, which induces obesity and insulin resistance, intravenous NPY *increases* insulin-induced whole-body glucose disposal, predominantly in skeletal muscle, with no effect on glucose metabolism in adipose tissue [60]. The explanation for these opposing data is unknown.

These studies provide evidence that the development of abdominal adiposity in insulin resistance may be mediated via the NPY pathway and Y2 receptors in abdominal fat. This is illustrated in Fig. (**3**). While generalized obesity may result from a combination of genes, which were advantageous in times of famine (an extension of the well known "thrifty gene' hypothesis) [61], it is similarly possible that increased central adiposity may preferentially develop in those with a hyper-responsive 'fight or flight' response, which would have then been an asset in protection of young from danger. These data suggest that insulin-induced SNS hyperresponsiveness, exacerbated by chronic 'stress', may be a potential mechanism contributing to the accumulation of abdominal fat tissue in genetically-prone insulin-resistant humans, via up-regulation of NPY and its Y2 receptor. If confirmed, antagonism of the Y2 receptor in visceral abdominal fat may represent a novel pharmacological pathway to reduce the accumulation of abdominal fat and prevent its metabolic complications.

CONCLUSIONS

In summary, we have reviewed evidence supporting the notion that impaired SNS responsiveness contributes to the pathogenesis of obesity in insulin-resistant individuals. However, whether early intervention to reduce sympathetic activity in insulin resistance will be an effective therapeutic pathway for preventing or delaying beta-



NPY = neuropeptide Y. PP = post-prandial. SNS = sympathetic nervous system. Y2R = Y2 receptor.

Fig. (3). Unifying hypothesis: Role of NPY in linking SNS overactivity to the development of abdominal (visceral) obesity in insulin resistance.

adrenergic down-regulation and desensitization and the development of obesity remains to be elucidated. The recent demonstration that stress-induced release of NPY from sympathetic nerves within abdominal adipose tissue may play a role in its accumulation, raises the possibility that hyperinsulinaemia-induced stimulation of the SNS promotes the accumulation of abdominal adipose tissue via increased expression of NPY and the Y2 receptor. Manipulations of the NPY-Y2 receptor pathway in abdominal adipocytes may represent a novel approach for reducing abdominal obesity and type 2 diabetes in genetically-prone humans with insulin resistance.

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ABBREVIATIONS

ANS	=	Autonomic nervous system
HF	=	High frequency
HRV	=	Heart rate variability
LF	=	Low frequency
MSNA	=	Muscle sympathetic nerve activity
SNS	=	Sympathetic nervous system
TNF	=	Tumour necrosis factor

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