

The peripheral sympathetic nervous system in human obesity

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Summary

The peripheral sympathetic nervous system is a key factor in the regulation of energy balance in humans. Differences in sympathetic nervous system activity may contribute to variations in 24 h energy expenditure between individuals. β -Adrenoceptors play a more important role than α -adrenoceptors in this regulation. The involvement of both β 1- and β 2-adrenoceptor subtypes has been demonstrated, the role of the β 3-adrenoceptor subtype is not yet clear. Normal or increased levels of sympathetic nervous system activity and reduced reactivity appear to be present in established obesity. Furthermore, the sensitivity for β -adrenoceptor stimulation is impaired in obesity. The blunted reactivity and sensitivity may contribute to the maintenance of the obese state. There are data to suggest that they may also play a role in the aetiology of obesity, because the impairments often remain after weight reduction. Furthermore, a negative correlation between baseline sympathetic nervous system activity and weight gain during follow-up has been found in Pima Indians. Recently, genetic evidence about the involvement of adrenoceptors in obesity has become available. Although the results of association and linkage studies on polymorphisms in the β 2-, β 3- and α 2-adrenoceptor genes are inconsistent, the functional correlates of some of these polymorphisms (changes in agonist-promoted down-regulation, protein expression levels, lipolytic sensitivity, basal metabolic rate, sympathetic nervous system activity) suggest that they may be important in the aetiology of obesity.

Keywords: adrenoceptor, energy expenditure, polymorphism, sensitivity.

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Introduction

Obesity is a rapidly growing problem with increasing impact on public health in many countries throughout the world. Effective prevention and treatment strategies are badly needed. The development of such strategies must be based on knowledge about the aetiology and pathophysiology of obesity.

The development of obesity is caused by a temporal disturbance of energy balance with energy intake exceeding energy expenditure. The sympathetic nervous system is involved in the regulation of both sides of the energy balance equation, but mainly the expenditure side. Genetic and environmental factors may influence the actions of

the sympathetic nervous system. Evidence suggesting that changes in sympathetic nervous system regulation of energy balance are involved in the aetiology and pathophysiology of human obesity will be reviewed here.

The peripheral sympathetic nervous system, together with the parasympathetic nervous system, plays an important role in the integrated response of many bodily functions in response to changes in the environment. The pre-ganglionic sympathetic neurones run within the spinal cord and exit the spinal cord in the thoracic and lumbar regions. The sympathetic ganglia are located close to the spinal cord and from these ganglia long post-ganglionic neurones originate that innervate the various target tissues by release of neurotransmitters from their varicosities. In most tissues noradrenaline

Table 1 Effects caused by stimulation of the different adrenoceptor subtypes in humans

Tissue	Effect	Adrenoceptor subtype				
		$\alpha 1$	$\alpha 2$	$\beta 1$	$\beta 2$	$\beta 3$
Heart	Rate			Increase	Increase	
	Force of contraction	Increase		Increase	Increase	
Skeletal muscle	Tremor				Increase	
	Glycogenolysis				Increase	
	Glucose uptake				Decrease	
	Na-K-ATPase				Increase	
Adipose tissue	Lipolysis		Decrease	Increase	Increase	Increase
Liver	Glycogenolysis	Increase			Increase	
	Gluconeogenesis				Increase	
Bronchi		Constrict			Dilate	
Blood vessels		Constrict	Constrict		Dilate	
Pancreas	Insulin secretion			Decrease	Increase	
	Glucagon secretion			Decrease	Increase	
Various tissues	Thermogenesis			Increase	Increase	

is the predominant neurotransmitter of the sympathetic nervous system. The adrenal medulla can be regarded as a specialized sympathetic ganglion. No post-ganglionic neurones originate from the adrenal medulla, but it releases hormones (mainly adrenaline) into the bloodstream instead.

In many physiological conditions the peripheral sympathetic nervous system does not respond as a whole, but regional variations in sympathetic activation are seen. In addition, sympathetic nervous system activation and adrenomedullary adrenaline secretion do not always run in parallel. In this review the term sympathetic nervous system will be used for the combination of the sympathetic nervous and adrenomedullary systems.

Receptors of the sympathetic nervous system

The receptors of the sympathetic nervous system can be divided in two main types, the α - and β -adrenoceptors, with at least two types of α -adrenoceptors and three types of β -adrenoceptors (Table 1). The α -adrenoceptors are located mainly in the blood vessels and play an important role in cardiovascular regulation. α -Adrenoceptors can also be found in adipose tissue, where they have an inhibitory effect on lipolysis, in the pancreas and in the liver. Presynaptic $\alpha 2$ -adrenoceptors are involved in the feedback control of noradrenaline release from the sympathetic nerve endings. β -Adrenoceptors have an important function in both cardiovascular and, especially, metabolic regulation. Nowadays the existence of three types of β -adrenoceptors, $\beta 1$ -, $\beta 2$ - and $\beta 3$ -adrenoceptors, in humans is acknowledged. The $\beta 1$ - and $\beta 2$ -adrenoceptor have been known since the 1960s (1). In the 1980s, with the development of relatively selective agonists, the existence of the third β -adrenoceptor became widely recognized (2). Soon after this, the gene

Table 2 Pharmacological characteristics of the human $\beta 1$ -, $\beta 2$ - and $\beta 3$ -adrenoceptors expressed in Chinese hamster ovary cells. Adapted from Strosberg and Piétri-Rouxel (8)

Agonist	Kact (nM)		
	$\beta 1$ -adrenoceptor	$\beta 2$ -adrenoceptor	$\beta 3$ -adrenoceptor
Noradrenaline	0.8 \pm 0.3	36 \pm 0.4	6.3 \pm 0.7
Adrenaline	2.7 \pm 0.8	2.2 \pm 0.5	49 \pm 5
Isoprenaline	0.19 \pm 0.04	2.5 \pm 0.8	3.9 \pm 0.4
CGP 12177A	antagonist	antagonist	139 \pm 44

Kact, activation constant for cyclic AMP stimulation.

encoding the $\beta 3$ -adrenoceptor from several species, including humans, was cloned, sequenced and was expressed in cell lines (3). The presence of $\beta 3$ -adrenoceptor mRNA has been demonstrated in human adipose tissue, gallbladder, stomach, small intestine, colon, prostate gland and (by some) in brain, but not in skeletal muscle, heart, liver, lung, kidney, thyroid and lymphocytes (4–6). Recently, the presence of $\beta 3$ -adrenoceptor protein has been demonstrated in human skeletal muscle (gastrocnemius) as well as the right atrium (7). The endogenous ligands of the β -adrenoceptors, the catecholamines noradrenaline and adrenaline, bind equally well to the $\beta 1$ -adrenoceptor; the $\beta 2$ -adrenoceptor has a higher affinity for adrenaline than for noradrenaline; and the $\beta 3$ -adrenoceptor has a higher affinity for noradrenaline than for adrenaline (2–8) (Table 2).

It can not be excluded that more adrenoceptor types and subtypes than those presently known are functional in humans. Certainty about the total number of adrenoceptor types and subtypes in humans will only be obtained when the whole human genome is known.

Table 3 Sympathetic nervous system activity changes in response to various physiological conditions as measured by different techniques. Adapted from Grassi and Esler (10)

Condition	Microneurography		NA spillover cardiac	renal	plasma NA concentration		HR spectral analysis ΔLFpower
	MSNA muscle	skin			venous	arterial	
Mental stress	0, ↑	↑	↑	↑	0	0	0, ↑
Vasovagal syncope	↓	?	↓	↓	↓	↓	0, ↑
Smoking	↓	↑	↑	?	0	↑	?
Low-salt diet	↑	?	0	↑	0	0	?
Postprandial phase	↑	?	0	↑	↑	0	?
Hyperinsulinaemia	↑	0, ↑	?	0	0	↑	?
Isometric exercise	↑	↑	↑	↑	↑	↑	0
Aerobic exercise	↑	?	↑	↑	↑	↑	↓
Exercise training	↓	?	0	↓	↓	↓	?
Diet-induced weight loss	↓	?	?	?	↓	?	?

NA, noradrenaline; MSNA, muscle sympathetic nerve activity; LF, low frequency band; ↑, increase; 0, no change; ↓, decrease;?, effect unknown.

Methods to assess sympathetic nervous system activity in humans

Several techniques to measure sympathetic nervous system activity in humans have been developed and used over the years. These techniques and their advantages and limitations have recently been reviewed (9,10). Plasma and urinary noradrenaline concentrations are still useful in assessing sympathetic neural function. However, the reproducibility of plasma and urinary noradrenaline measurements is not very high. In addition, plasma and urine concentrations not only depend on sympathetic nervous system activity, but also on clearance of noradrenaline from the circulation which may also vary. The development of tracer techniques for the measurement of noradrenaline spillover into the plasma has largely solved this problem. This technique also allows for the estimation of regional sympathetic neural function, which is an important advantage because the sympathetic nervous system shows discrete regional activation patterns under many physiological and pathophysiological conditions. A limitation of this technique, however, is that spillover measurements are influenced by competing disposition mechanisms, such as blood flow and neuronal re-uptake (9,10). Technical developments have allowed direct intraneural recording of sympathetic nerve traffic by microneurography. Although this technique is reproducible, its application is limited to peripherally accessible nerves and, therefore, allows the recording of sympathetic nerve traffic to skeletal muscle or skin only. Power spectral analysis of heart rate variability is a technique to identify superimposed rhythms producing cyclic variations in heart rate. The low frequency variability of the spectrum derives in part from the influence of the cardiac sympathetic nerves. Nevertheless, a relationship between changes in low-frequency heart rate spectral power and rates of noradren-

aline spillover from the heart is absent in many conditions (10).

In Table 3 the responses of the sympathetic nervous system to different conditions, as measured by different techniques, are summarized. None of the techniques can be regarded as the ‘standard’ for the measurement of sympathetic nervous system activity. Combining several measurement techniques will usually give a more complete picture (9,10). It is also important to stress that most techniques currently preferred to measure sympathetic nervous system activity, microneurography and noradrenaline spillover, do not include measurement of the activity of the adrenal medulla, which often has an activation pattern distinct from the nervous system (Table 4) and plays an important role in the regulation of metabolism.

The peripheral sympathetic nervous system and energy balance

The energy balance equation tells us that changes in body energy stores occur when energy intake and energy expenditure are not in balance. The sympathetic nervous system may be one of the systems that is involved in the integrative response to changes in either aspect of energy balance, energy expenditure or energy intake, in order to maintain long-term energy balance and, probably more importantly, prevent depletion of body energy stores. The influence of the sympathetic nervous system on energy expenditure has been studied extensively. Much less is known about its influence on energy intake.

Sympathetic nervous system and energy intake

Most available information about the effect of the peripheral sympathetic nervous system activity on food intake

Table 4 Adrenal medullary and sympathetic nerve responses to different physiological stimuli and in different diseases. Adapted from Grassi and Esler (10)

Condition	Sympathetic nerves MSNA	total NA spillover	Adrenal medulla plasma A conc	A secretion rate
Vasovagal syncope	↓	↓	↑	?
Short-term fasting	0	↓	↑	?
Smoking	↓	0	↑	?
Tricyclic antidepressant	↓	↓	↑	↑
Ageing	↑	0, ↑	0	↓
Cardiac failure	↑	↑	0, ↑	0

NA, noradrenaline; A, adrenaline; MSNA, muscle sympathetic nerve activity; ↑, increase; 0, no change; ↓, decrease; ?, effect unknown

comes from animal studies. Indices of peripheral sympathetic nervous system activity, such as noradrenaline turnover in brown adipose tissue (BAT) or firing of the sympathetic nerves innervating BAT, show an inverse relationship with food intake in experimental animals (11). There is not much experimental evidence for such a relationship in humans. Raben and co-workers (12) describe a negative relationship between changes in plasma noradrenaline concentration and changes in hunger score after different types of meals. However, many other parameters in that study show a similar strong negative relationship with hunger scores. Whether the variations in peripheral sympathetic activity are the cause of variations in food intake or just a concurrent effect of other processes in the central nervous system which regulate food intake is not clear. The most direct evidence that peripheral sympathetic activity regulates food intake comes from animals with a targeted disruption of the β_3 -adrenoceptor gene in white adipose tissue. In wildtype animals administration of β_3 -adrenoceptor agonists acutely reduces food intake, but this effect is lost in the β_3 -adrenoceptor knock out animals (13).

These results may indicate that high peripheral sympathetic nervous system activity, which is associated with high energy expenditure, is associated with reduced energy intake. Such a relationship is understandable if the increased sympathetic nervous system activity and energy expenditure occur as the acute response to a meal or during exercise. However, the relationship between sympathetic nervous system activity and energy intake under various conditions clearly needs further study in humans.

Sympathetic nervous system and energy expenditure

The relationship between daily energy expenditure and activity of the sympathetic nervous system has not been well studied. In two studies (14,15) a positive correlation between 24h energy expenditure, adjusted for body size and body composition, and measures of sympathetic

nervous system have been found during a stay in a respiration chamber, but in another study no significant relationship was present (16). Furthermore, β -adrenoceptor blockade did not reduce 24h energy expenditure significantly (17,18). Based on the study by Toubro and co-workers, (15) Astrup and Macdonald (9) calculated that the difference in 24h energy expenditure between subjects with high and low sympathetic nervous system activity was approximately 750 kJ day^{-1} . Since these results were obtained in the respiration chamber, with fixed diet and physical activity, bias by these factors is excluded. If these data can be reproduced, they suggest that interindividual differences in sympathetic nervous system activity induce variations in energy expenditure that may be relevant to energy balance.

The influence of sympathetic nervous system activity on resting metabolic rate is relatively small. Administration of the β -adrenoceptor blocking agent propranolol reduces resting metabolic rate by 3–4% (14–19). After a meal, energy expenditure, as well as sympathetic nervous system activity, rise. The increase in energy expenditure is positively correlated with the increase in the appearance rate of noradrenaline (20). The quantitative contribution of the sympathetic nervous system to the thermic effect of food (on average 30–40%) depends strongly on the size and the nutrient composition of the meal, particularly on the carbohydrate content which is positively correlated with the sympathetic nervous system response (9). Physical activity is associated with a marked activation of the sympathetic nervous system. However, β -adrenoceptor blockade does not significantly reduce energy expenditure during exercise (van Aggel-Leijssen and co-workers, unpublished data). The increased energy expenditure seen after exercise also does not appear to be related to sympathetic nervous system activation, since it cannot be blocked by β -adrenoceptor antagonists (21).

The different adrenoceptor subtypes contributing to sympathetic nervous system-mediated thermogenesis have been studied by the administration of antagonists with

varying receptor subtype selectivity or by the administration of selective agonists. α -Adrenoceptors do not appear to play a role in the thermogenic effect of sympathetic nervous system activity (19). Stimulation of β 1- as well as β 2-adrenoceptors cause increased energy expenditure (19–23). Whether the β 3-adrenoceptor plays a significant role in human thermogenesis remains unclear. Studies aimed at resolving this question have yielded contradictory results. Blaak and co-workers (19) studied the contribution of β 3-adrenoceptor to thermogenesis induced by infusion of catecholamines and found no evidence for non- β 1 + 2-adrenoceptor-mediated thermogenesis. Liu and co-workers (24), on the other hand, showed that the thermogenic response to ephedrine was not fully blocked after a low dose of β 1 + 2-adrenoceptor antagonist, which completely inhibited all β 1- and β 2-adrenoceptor-mediated responses. The discrepancies between these studies may be due to the fact that different modes of stimulation were used, either infusion of catecholamines (19) or administration of ephedrine (24), an indirect sympathomimetic agent that increases the release of noradrenaline into the synaptic cleft, which may have induced different patterns of adrenoceptor activation. Studies by Wheeldon and co-workers (25) and Schiffelers and co-workers (26), investigating a possible β 3-adrenoceptor component in the thermogenic effect of isoprenaline, came to opposing conclusions. The definitive answer to whether or not part of the sympathetic nervous system-induced thermogenesis is mediated by β 3-adrenoceptors has to await the development of selective β 3-adrenoceptor antagonists for clinical use in humans.

Sympathetic nervous system and substrate oxidation

Apart from its thermogenic effect, the β -adrenergic sympathetic nervous system also influences the composition of the substrate mix that is oxidized. During β -adrenoceptor stimulation by infusion of the nonselective agonist isoprenaline the relative contribution of fat oxidation to total energy expenditure increases, reflected by a significant decrease of the respiratory exchange ratio (27,28). β -Adrenoceptor blockade by propranolol has been shown to reduce 24 h fat oxidation (17,18). The increase in relative fat oxidation is found after selective stimulation of β 1-adrenoceptors by dobutamine (22) as well as after selective β 2-adrenoceptor stimulation by salbutamol (23) (Fig. 1).

Sympathetic nervous system activity in human obesity

Different methodological approaches have been applied to the study of sympathetic nervous system activity in obesity. In many studies plasma or urine catecholamine levels have been measured. Young and Macdonald (29) reviewed the

available data in 1992 and the review was updated by Macdonald (30) in 1995. Noradrenaline concentrations were lower in obese than in lean subjects in 14 studies, equal in 21 studies and higher in obese than in lean subjects in 11 studies. Microneurographic recordings of the peroneal nerve, innervating the skeletal muscle circulation, show a strong positive relationship with body weight, body mass index or percentage body fat (31–34). Regional noradrenaline spillover measurements suggest that sympathetic nervous system activity in obesity is normal for the whole body (also for adrenaline spillover), but increased in the kidneys and reduced in the heart (35,36). In contrast, whole body noradrenaline spillover was positively correlated with body fatness in another study (37). Analysis of heart rate variability in obese subjects suggests that they have increased sympathetic nervous system activity (38). There is some evidence that the response of the sympathetic nervous system to various physiological stimuli (underfeeding, hyperinsulinaemia; cold exposure) is blunted in those who are obese (9,33,39).

The data on activity of the sympathetic nervous system in obesity show considerable heterogeneity, which may be related to the influence of diet and physical activity on basal sympathetic tone (9). However, in general, the data point to a normal or increased level of sympathetic nervous system activity in most tissues, while responsiveness may be blunted, in established obesity. A high basal level of sympathetic nervous system activity in obesity may be associated with the development of comorbidities such as insulin resistance, dyslipidemia and hypertension.

Sensitivity to sympathetic nervous system activation in human obesity

As indicated above, obesity appears to be associated with normal or increased sympathetic nervous system activity in most tissues and a blunted sympathetic nervous system reactivity in certain physiological conditions. There are also many studies showing that the sensitivity to a certain level of sympathetic nervous system activity is reduced in obesity. Blaak and co-workers (27) reported that the dose of the β -adrenoceptor agonist isoprenaline needed to increase energy expenditure by 15% was positively correlated with percentage body fat, suggesting a reduced thermogenic response to humoral β -adrenoceptor stimulation in the obese. Subsequent studies showed that it was the β 2-adrenoceptor-mediated response rather than the β 1-adrenoceptor-mediated response that was impaired (23) (Fig. 1). Apart from the thermogenic response, other sympathetic nervous system-induced responses also seem to be blunted in obesity. Changes in vascular resistance with noradrenaline infusion are positively correlated with body mass index (40). Reynisdottir and co-workers (41) found a 10-fold decreased lipolytic sensitivity to noradrenaline

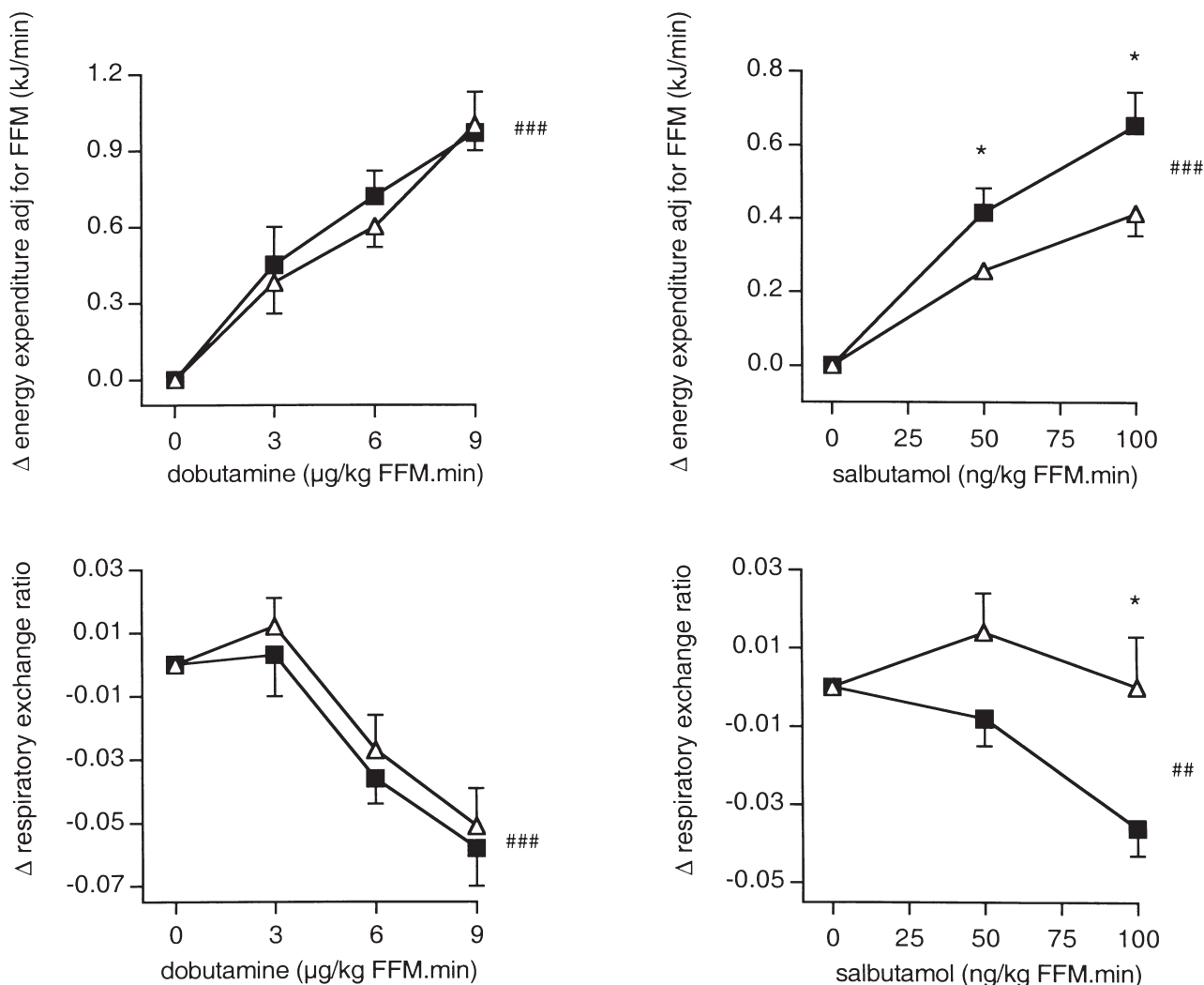


Figure 1 Changes in energy expenditure and respiratory exchange ratio during β_1 -adrenoceptor stimulation with dobutamine and β_2 -adrenoceptor stimulation with salbutamol in lean (black squares) and obese (triangles) men. ### $P < 0.001$ increase from baseline; * $P < 0.05$ lean vs. obese.

per cell in abdominal subcutaneous fat cells from upper body obese women compared to non-obese women. The decrease was associated with a lower β_2 -adrenoceptor density in the adipocytes from upper-body obese women. Evidence for a reduced lipolytic sensitivity in obesity is also found in *in vivo* studies (23–27,42,43). However, when expressed per kg fat-free mass rather than per fat mass, there appears to be no difference in lipolysis between lean and obese individuals. Wolfe and co-workers, (42) therefore, concluded that the blunted lipolytic response in obese subjects may simply reflect the decreased lipolytic demand to meet the energy requirements of the lean body mass.

Several studies conclude, based on changes in respiratory exchange ratio, that the increase in relative fat oxidation after β -adrenergic stimulation is blunted in the obese (23,27,28) (Fig. 1). Webber and co-workers (43), on the other hand, found that palmitate oxidation, measured by tracer

technique, did not change in lean subjects but was significantly reduced in obese subjects during adrenaline infusion.

The impaired sympathetic nervous system reactivity and reduced sensitivity to sympathetic nervous system stimulation in those who are obese may contribute to maintenance of the obese state. Whether these factors also play a role in the aetiology of obesity will be discussed in the next paragraph.

Role of the sympathetic nervous system in the aetiology of human obesity

Although a normal to high level of sympathetic nervous system activity is most common in obesity, this does not exclude the possibility that low sympathetic nervous system activity, reactivity and/or sensitivity play a role in the aetiology of obesity. However, at the moment there is not much

direct evidence to support such a role, most of the evidence is indirect.

Tataranni and co-workers (44) showed that the baseline urinary excretion rate of noradrenaline, as an index of sympathetic nervous system activity, was negatively correlated with body weight gain over a 3.3 ± 2.1 y follow-up period in Pima Indians, a population with a high incidence of obesity and type 2 diabetes mellitus. Baseline adrenaline excretion correlated negatively with changes in waist-to-thigh circumference. Hellström and co-workers (45) reported resistance of lipolysis to catecholamines, at least partly due to impaired function of hormone-sensitive lipase, in abdominal subcutaneous fat cells of non-obese subjects with a family trait for obesity (at least one of the first-degree relatives having a BMI = 27 kg m^{-2}). Another study showed that children in the dynamic phase of obesity development have an impaired lipolytic response to adrenaline infusion (46). Schifflers and co-workers (47) recently found that a blunted lipolytic response to a β -adrenoceptor agonist *in vivo* is associated with an increased fat mass, even in the absence of obesity.

In post-obese women, lower basal adrenaline concentrations have been found than in never-obese women. In addition, the adrenaline response to feeding was blunted in the post-obese women. Noradrenaline concentrations did not differ (48). On the other hand, Buemann and co-workers (18) reported that the β -adrenoceptor antagonist propranolol reduced 24 h energy expenditure in reduced-obese women (-2.7%), but not in never-obese women (-0.8%), suggesting increased sympathetic nervous system activity or sensitivity in reduced-obese women.

The results of weight loss studies are also inconclusive. Blaak and co-workers (49) found that weight reduction significantly improved the thermogenic response to β -adrenoceptor stimulation and lowered basal plasma noradrenaline concentrations in obese men. Weight loss also reversed the reduced β 2-adrenoceptor lipolytic sensitivity in upper-body obese women (50). Furthermore, Mauriège and co-workers (51) showed that weight loss improved the sensitivity of lipolysis to β -adrenoceptor and β 2-adrenoceptor stimulation, the effect being greater in abdominal than in femoral adipocytes. The α 2-adrenoceptor antilipolytic sensitivity was reduced by weight loss (51). On the other hand, the reduced lipolytic response to isoprenaline infusion *in vivo* in obese men was not improved by weight loss (52). Weight loss also diminished the β -adrenoceptor-mediated increase in fat oxidation during exercise in obese men, suggesting a diminished sensitivity to β -adrenoceptor stimulation, since plasma catecholamine concentrations during exercise were not affected by weight loss (Van Aggel-Leijssen and co-workers unpublished data). It is possible that the discrepancies between the results of weight loss studies and those in post- and pre-obese subjects are due to the continuing influence of the

energy-restriction period in the weight loss studies, even though body weight has been stable for a limited period of time (2–4 weeks). However, most evidence supports a role for low sympathetic nervous system activity and/or sensitivity in the aetiology of obesity.

Genetic evidence for the involvement of adrenoceptors in human obesity

In the general population the excessive accumulation of fat is likely to be due to an interaction between genetic and environmental factors. A large number of candidate genes for obesity has been proposed, but until now no consistent associations with different obesity-related phenotypes have been observed (53). The adrenoceptors genes can be regarded as candidate genes for obesity, because of the important role the sympathetic nervous system plays in the regulation of energy metabolism and the impairments in sympathetic nervous system activity and sensitivity that have been found in relation to human obesity as described above. Most of the evidence for a role of adrenoceptors in the genetics of obesity comes from studies on polymorphisms in the β 2-, the β 3-, and α 2-adrenoceptor genes.

β 2-Adrenoceptor gene

The gene for the β 2-adrenoceptor is located on chromosome 5 q31–32. Although at least four polymorphisms in the β 2-adrenoceptor gene are known (54), the two most common ones have been studied in relation to obesity: at codon 16 (Arg16Gly) and at codon 27 (Gln27Glu). Both polymorphisms are located at the amino-terminal site of the receptor. Several functional consequences of these polymorphisms have been described. When the Gly16 variant of the receptor is transfected into Chinese hamster fibroblasts, it shows enhanced agonist-promoted down-regulation compared with the wildtype receptor. β -Agonist-induced cyclic AMP production is normal (54). Tan and co-workers (55) reported that the ventilatory responses to a β 2-adrenoceptor agonist were reduced in asthmatics carrying the Gly16 allele, which might indicate decreased expression of the receptor. On the other hand, Large and co-workers (56) showed that women carrying the Gly16 variant had increased sensitivity of their abdominal subcutaneous fat cells for β 2-adrenoceptor stimulation but normal β 2-adrenoceptor expression. Another study showed that diet plus exercise induced weight loss was greater in Japanese obese women carrying the Gly16 variant than in those without the mutation (7.6 vs. 5.5 kg in 3 months) (57). The transfected mutant Glu27 receptor is resistant to agonist-promoted down-regulation compared with the wildtype receptor and has normal sensitivity for β -adrenoceptor agonists (54). The Glu27 variant is not associated with changes in *in vitro* subcutaneous fat

cell lipolytic sensitivity to β 2-adrenoceptor stimulation in Swedish women (56).

In an early study by Oppert and co-workers (58) in members of the Québec Family Study cohort, no association or linkage of the β 2-adrenoceptor/BanI restriction fragment length polymorphism with BMI or markers of fat distribution was detected. Since then, a number of studies have investigated the association between the Gln27Glu and the Arg16Gly polymorphisms and obesity. In a group of Swedish women of varying body mass index the Glu27Glu variant was significantly more frequent in obese (BMI > 27 kg m⁻²) than in non-obese women (56). In contrast, the frequency of the Glu27 allele was significantly lower in obese Swedish males than in non-obese males (59). In Japanese subjects the Glu27 allele was significantly more frequent in the obese than in the non-obese, in men as well as women (60,61). In another study the interaction between the Gln27Glu polymorphism and physical activity with respect to body mass was studied in a representative sample of the Northern French population. Surprisingly, physically inactive Gln27Gln homozygous males had an increased BMI compared with inactive carriers of the Glu27 allele (27.2 vs. 25.2 kg m⁻², $P < 0.0001$) or active men irrespective of their genotype. No interaction between genotype and physical activity was found in women (62).

The Arg16Gly polymorphism was not associated with obesity in a group of Swedish women (56). Data from the Japanese population on the association between the Arg16Gly polymorphism and obesity are inconsistent. Ishiyama-Shigemoto and co-workers (60) showed that the Gly16 allele was less common in obese Japanese women than in non-obese women, while no association between the Arg16Gly polymorphism and obesity was found in males. On the other hand, Sakane and co-workers (57) found that the Gly16 variant was not associated with higher BMI in Japanese women.

Yamada and co-workers (63) reported that two polymorphisms in the untranslated 5'-leader cistron of the β 2-adrenoceptor, encoding a leader protein which inhibits β 2-adrenoceptor expression, are associated with greater BMI and higher serum triglyceride concentrations. These two polymorphisms are tightly linked and also show linkage with the codon 16 and, especially, codon 27 polymorphisms. A linkage analysis of microsatellite markers on chromosome 5 q31-32, where the β 2-adrenoceptor is located, in 264 families showed no linkage with BMI (64).

β 3-Adrenoceptor

The human β 3-adrenoceptor gene is located on chromosome 8. A polymorphism at codon 64 of the gene (Trp64Arg), located in the first intracellular loop of the receptor, has attracted attention from obesity researchers.

In 1995 the association of the Trp64Arg polymorphism with earlier onset type 2 diabetes, features of insulin-resistance and tendency to weight gain was first reported (65-67). Two additional polymorphisms have been identified recently (at nucleotide positions 1856 and 3139), in almost complete linkage disequilibrium with the Trp64Arg polymorphism at position 827 (68).

The functional consequences of the Trp64Arg polymorphism have been studied quite extensively, with mostly negative results. Trp64Arg mutants of the human β 3-receptor expressed in Chinese hamster ovary (CHO) cells were shown to be pharmacologically and functionally (cAMP production) indistinguishable from wildtype receptors (69). On the other hand, Piétri-Rouxel and co-workers (70) showed reduced maximal cAMP production in response to various β 3-agonists, noradrenaline, adrenaline, isoprenaline and CGP 12177 A, in two different cell types (CHO and human HEK cells) with stable expression of the Arg64 variant of the human β 3-adrenoceptor compared with the Trp64 variant. The first *in vivo* human studies on lipolytic sensitivity were unable to demonstrate functional differences between carriers of the various alleles. In Pima Indians isoprenaline-induced lipolysis in subcutaneous abdominal fat was unchanged in Arg64 homozygotes as compared with Trp64 homozygotes (as determined by microdialysis) (71). These results were supported by data on *in vitro* basal and isoprenaline-induced lipolysis in abdominal subcutaneous adipocytes of a larger group of Pima Indians (72). Fasting plasma NEFA concentration, lipid oxidation and NEFA rate of appearance also did not differ between variants in this study (72). Li and co-workers (73) showed that the *in vitro* sensitivity and responsiveness of visceral adipocytes to noradrenaline or CGP 12177 were similar in Swedish non-obese or obese subjects carrying or not carrying the Arg64 variant. A more recent study from the same group of investigators, however, showed that CGP12177-stimulated lipolysis in visceral adipocytes was 10-fold decreased in β 3-Arg haplotype subjects (-7.8 vs. -8.8 log mol/L, $P = 0.01$) (68). Another study also showed lower sensitivity and responsiveness of omental fat cells to β 3-adrenoceptor stimulation (by L-755,507, a new β 3-adrenoceptor agonist) in Arg64 homozygotes ($n = 4$) than in wildtype Trp64 homozygotes ($n = 8$). However, this difference was not evident with isoprenaline- or CGP 12177-induced lipolysis (74). Because of the small number of subjects, the as yet incompletely established β 3-selectivity of L-755,507, and the absence of a difference with noradrenaline and CGP 12177, these results have to be interpreted with caution.

Several studies have looked at the relationship between the β 3-adrenoceptor polymorphism and parameters of energy expenditure. Walston and co-workers (65) reported a tendency for a lower resting metabolic rate in Pima Indians carrying the Arg mutation. In the Québec Family

Study no difference in resting metabolic rate was found between subjects carrying the Arg64 allele and Trp64 homozygotes (75). However, sibpair linkage analysis in the same population revealed weak linkage between the Trp64Arg polymorphism and resting metabolic rate (75). A significantly lower resting metabolic rate was reported in obese male and female Finns carrying the Arg64 variant (76). On the other hand, Tchernof and co-workers (77) found no relationship between energy expenditure and the Trp64Arg polymorphism in obese US women, but never-obese women with the Arg64 variant had a reduced resting metabolic rate. These authors indicate that the presence of obesity may have masked the effect of the Trp64Arg polymorphism on resting metabolic rate in obese women.

A recent study in normal weight Japanese subjects reported a lower basal sympathetic nervous system activity in subjects carrying the Arg64 variant of the β 3-adrenoceptor gene, based on R-R interval spectral analysis. Upon standing the difference disappeared, possibly indicating a greater responsiveness of the sympathetic nervous system in subjects with the Arg64 variant (78).

Studies on the association between the Trp64Arg polymorphism and obesity-related phenotypes have yielded inconsistent results (3–81). Recently published, large studies in Finland ($n = 1725$) and the Netherlands ($n = 600$) did not find an association between the Trp64Arg polymorphism and measures of obesity (82,83). Studies relating the β 3-adrenoceptor polymorphism to weight gain or capacity to lose weight have also yielded inconsistent results (81). A quantitative linkage analysis of the β 3-adrenoceptor and obesity using 12 markers spanning a 57-cM region surrounding the β 3-adrenoceptor gene on chromosome 8 in families of Mexican-American ancestry showed peak evidence for linkage <3cM from the estimated position of the β 3-adrenoceptor gene. The observed linkage could, however, not be attributed to the Trp64Arg polymorphism. This might be explained by the presence of an other important polymorphism in the β 3-adrenoceptor gene or in other genes in this region, or by the influence of the β 3-adrenoceptor gene in combination with polymorphisms at other genes (84).

Several studies indicate synergistic effects of polymorphisms in the UCP (uncoupling protein)-1 and β 3-adrenoceptor genes with respect to basal metabolic rate (85), weight gain in morbid obesity (86) and weight loss (87).

α 2-Adrenoceptors

A restriction fragment length polymorphism (DraI) in the α 2A-adrenoceptor gene, located on chromosome 10, showed significant linkage with fat distribution in the Québec Family Study (58). The α 2A-adrenoceptor is involved in the regulation of adipose tissue lipolysis.

Recently, a polymorphism (three amino acid deletion) in the α 2B-adrenoceptor gene, located on chromosome 2, has been demonstrated to be associated with reduced basal metabolic rate in obese, non-diabetic Finns (88). The distribution and physiological functions of this receptor subtype is largely unknown, but studies in mice indicate that activation of α 2B-receptors in blood vessels causes vasoconstriction. The variant form might be associated with increased vasoconstriction, because the mutant receptor is less sensitive to phosphorylation-mediated desensitization. The investigators, therefore, suggest that this may cause altered distribution of blood flow away from metabolically active tissues (88).

In conclusion, association and linkage studies of polymorphisms in the adrenoceptor genes with obesity have so far yielded conflicting results. Lack of consistency may be due to low statistical power because of small sample sizes, the study of different, often selected populations, and interference by gene-gene and/or gene-environment interactions. Functional consequences do, however, suggest that these polymorphisms may in some way be implicated in obesity, since some functional consequences that have been found (increased desensitization, reduced lipolytic sensitivity, reduced basal metabolic rate, reduced sympathetic nervous system activity) may be important in relation to the accumulation of body fat and, thus, the development of obesity.

Conclusion

The sympathetic nervous system is one of the key factors involved in the regulation of energy balance in humans. Differences in sympathetic nervous system activity may contribute to variations in 24 h energy expenditure between individuals. These effects appear to be mediated by β -adrenoceptors rather than α -adrenoceptors. Both β 1- and β 2-adrenoceptor subtype involvement has been demonstrated, but the role of the β 3-adrenoceptor subtype is not yet clear. Although study results show considerable heterogeneity, most data suggest that established obesity is associated with normal or increased levels of sympathetic nervous system activity in most tissues and that there is some evidence for reduced sympathetic nervous system reactivity. Furthermore, the sensitivity for β -adrenoceptor stimulation appears to be impaired in obesity. The blunted reactivity and sensitivity may contribute to the maintenance of the obese state. There are also data to suggest that they may play a role in the aetiology of obesity, although most of the evidence is indirect, coming from weight loss studies and post-obese subjects. So far, only one study has investigated the relationship between baseline sympathetic nervous system activity and weight gain during follow-up (44). This study, in Pima Indians, showed a significant negative correlation, suggesting that low sympathetic nervous system

activity was associated with body weight gain. Recently, genetic evidence about the involvement of adrenoceptors in obesity has become available. Although the result of association and linkage studies on polymorphisms in the β_2 -, β_3 - and α_2 -adrenoceptor genes are inconsistent, the functional correlates of some of these polymorphisms (changes in agonist-promoted down-regulation, protein expression levels, lipolytic sensitivity, basal metabolic rate, sympathetic nervous system activity) suggest that they may be important in the aetiology of obesity.

In conclusion, evidence is accumulating that low sympathetic nervous system activity and reactivity and/or reduced sensitivity to sympathetic stimulation may play a role in the development and maintenance of obesity. Strategies that improve or compensate for these defects may contribute to the prevention and treatment of obesity.

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