STUDIES OF LIPOLYSIS AND NEUROENDOCRINE RHYTHMS IN CLUSTER HEADACHE

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ABSTRACT

Cluster headache (CH) is characterised by excruciating, unilateral headache attacks, which in the episodic form appear in clusters for weeks or even months. The headache attacks are often accompanied by ipsilateral, cranial autonomic symptoms supporting a local peripheral sympathetic deficit and a parasympathetic discharge. CH attacks are also associated with various cardiovascular changes indicating a systemic, autonomic disturbance as well, although the reports are contradictory. The etiology of CH is not known but a hypothalamic involvement is strongly suggested. Since hypothalamus is of great importance for the integration of autonomic nervous system signals, a disturbance in this area provides a possibility of a systemic, autonomic dysfunction of central origin in CH. The overall objective with this project was to focus on a better understanding of autonomic and neuroendocrine functions in CH. As a metabolic marker of systemic autonomic activity, adipose tissue lipolysis was studied, a metabolic function that is directly and indirectly regulated by hypothalamus.

In study I we investigated nocturnal lipolysis in CH patients as a marker for nocturnal sympathetic function compared to healthy controls. We found diminished nocturnal lipolysis in CH patients, both in active period and in remission. In addition, patients in remission showed an altered nocturnal lipolysis rhythm. These findings reflect for the first time a metabolic disturbance in CH and may be a result of a systemic sympathetic dysregulation, possibly at a hypothalamic level.

Most of the CH patients are heavy smokers. In study II we investigated to which extent smoking habits affect lipolysis in habitual smokers. We did not find any differences in nocturnal lipolysis between healthy smokers, after short-term tobacco withdrawal, and healthy non-smokers, which indicates that our finding of diminished nocturnal lipolysis in CH patients is not explained by smoking habits.

The lipolytic activity may be affected by disturbed β-receptor function. In study III we showed that diminished lipolysis in CH is not caused by defective β-receptors in adipose tissue, at least not in remission. On the contrary, β-receptors in adipose tissue appeared to be up-regulated, which may be a sign of decreased sympathetic tone and in support of a systemic sympathetic dysregulation in CH.

In study IV we investigated if a diminished lipolysis in CH remission could be a result of altered nocturnal secretion of noradrenaline, growth hormone (GH), insulin or cortisol, hormones with a potential effect on lipolysis and which at least partly are regulated by hypothalamic activity. We found altered GH concentrations during the early part of the night in CH but a normal secretion of noradrenaline, insulin and cortisol compared to controls. We suggested that the altered nocturnal GH pattern in remission in part may explain the altered nocturnal lipolysis in CH and support a permanent hypothalamic disturbance in CH.

Keywords: Cluster headache, autonomic nervous system, hypothalamus, lipolysis, microdialysis
LIST OF PUBLICATIONS

This thesis is based on the following articles and will be referred to by their Roman numerals:


IV. **Laudon Meyer E**, Marcus C, Waldenlind E. Nocturnal secretion of growth hormone, noradrenaline, cortisol and insulin in cluster headache remission *Manuscript*
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<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropin</td>
</tr>
<tr>
<td>AGP</td>
<td>$\alpha$-glycerophosphate</td>
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<td>ATBF</td>
<td>Adipose tissue blood flow</td>
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<td>CGRP</td>
<td>Calcitonin-gene-related peptide</td>
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<td>CH</td>
<td>Cluster headache</td>
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<tr>
<td>CRH</td>
<td>Corticotropin releasing hormone</td>
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<tr>
<td>FFA</td>
<td>Free fatty acid</td>
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<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
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<tr>
<td>GH</td>
<td>Growth hormone</td>
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<tr>
<td>GHRH</td>
<td>Growth hormone releasing hormone</td>
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<tr>
<td>GnRH</td>
<td>Gonadotropin releasing hormone</td>
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<tr>
<td>HPA-axis</td>
<td>Hypothalamic-pituitary-adrenal axis</td>
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<tr>
<td>HSL</td>
<td>Hormone sensitive lipase</td>
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<tr>
<td>IGF-1</td>
<td>Insulin growth factor-1</td>
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<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
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<tr>
<td>LHRH</td>
<td>Luteinizing hormone releasing hormone</td>
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<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>NA</td>
<td>Noradrenaline</td>
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<td>NO</td>
<td>Nitric oxide</td>
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<td>NPY</td>
<td>Neuropeptide Y</td>
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<td>PET</td>
<td>Positron emission tomography</td>
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<tr>
<td>PVN</td>
<td>Paraventricular nucleus</td>
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<tr>
<td>RVLM</td>
<td>Rostral-ventral-lateral medulla</td>
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<tr>
<td>SCG</td>
<td>Superior cervical ganglion</td>
</tr>
<tr>
<td>SCN</td>
<td>Suprachiasmatic nucleus</td>
</tr>
<tr>
<td>TAC</td>
<td>Trigemino autonomic cephalalgia</td>
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<tr>
<td>TG</td>
<td>Triglycerides</td>
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<tr>
<td>TRH</td>
<td>Thyrotropin releasing hormone</td>
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<tr>
<td>TSH</td>
<td>Thyrotropin</td>
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<tr>
<td>VIP</td>
<td>Vaso-active intestinal polypeptide</td>
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1 BACKGROUND

1.1 INTRODUCTION

One of my first patients with severe, chronic cluster headache is a musician. He told me once, that he never had suffered a headache attack during a performance but often shortly after a concert. Over the years, several other patients have witnessed about similar situations, freedom from attacks during intense mental or physical activity and recurrence of attacks while relaxing afterward and during sleep. Could a high sympathetic activity, like during mental stress, protect from developing attacks? Or on the contrary, do the attacks tend to develop during a situation of low sympathetic activity? These questions raised my interest for starting this project, which extended to involve breakdown of adipose tissue, receptors and different hormones in the blood, far away from the musician at the stage. Finishing this project, I am still not able to answer all these specific questions, but hopefully this thesis will bring some new knowledge into this area. I want to emphasize that the “musician” was the very first patient included in this project.

Cluster headache (CH) is classified as a primary headache syndrome and characterised by excruciating, unilateral headache attacks, which in the episodic form appear in clusters for weeks or even months. CH attacks are often accompanied by autonomic symptoms indicating a sympathetic and parasympathetic nervous system dysfunction. The role of the autonomic disturbance in CH is unclear. Are the autonomic symptoms secondary phenomena to pain and disturbed sleep patterns or is there a primary, autonomic disturbance, which during certain circumstances could be involved in initiating the attacks or even the cluster periods? Additionally, is the autonomic disturbance of peripheral or central origin? This thesis has focused on autonomic and neuroendocrine functions in CH.
1.2 CLUSTER HEADACHE

1.2.1 Clinical picture
The International Headache Society (IHS) has established operational clinical diagnostic criteria for primary and secondary headache disorders, first published 1988 and revised in 2004. The revised criteria for episodic and chronic CH are shown in table 1 (1). CH is classified as a trigeminal autonomic cephalalgia (TAC), a group consisting of primary, short-lasting headache syndromes with marked autonomic symptoms.

CH is a very painful condition, so severe that it sometimes is called “suicide headache”. In the episodic CH, the attacks come in clusters of varying duration, so called cluster periods or bouts. Most often the cluster periods last from three to six weeks. They are separated by pain-free periods from one month to several years, i.e. the remission phase. In previous definitions of cluster headache at least two cluster periods were required for the diagnosis, but it is possible that a few patients suffer from one period only during their lifetime (2). CH is predominantly an episodic disease, but in 10-20 % of the patients the condition is chronic (3), meaning that cluster attacks continue to recur for twelve months to several years without remission.

The pain is excruciating, sometimes described as “a red-hot knife being turned around”. The pain is situated behind and around one eye radiating toward the temple and/or to the upper cheek(4). It is strictly unilateral and in general hitting in the same side of the head from attack to attack. The attacks last for 15 to 180 minutes and appear with a frequency of one every other day to eight per day.

CH attacks are in 97 % of the patients accompanied by at least one ipsilateral cranial autonomic symptom. Ipsilateral miosis and ptosis are present during attacks in about 60% of the patients (5), suggesting a diminished sympathetic activity. In some patients these symptoms persist even between the cluster periods. Reddening of the eye, lacrimation, nasal congestion and secretion, indicate a parasympathetic neuronal discharge.

During the attacks the patients have a sense of restlessness which forces them to move around rather than stay in bed.
During cluster period, but not during remission, headache attacks may be provoked by various vasodilator stimuli, such as alcohol, histamine or nitroglycerin (6).

A remarkable feature of the CH symptomatology is the clock-wise regularity in which the headache attacks as well as the active periods tend to appear. The patients often know at which time of the day or night the headache attacks will show up and also at

<table>
<thead>
<tr>
<th>Diagnostic criteria Cluster Headache</th>
<th>Episodic Cluster Headache</th>
<th>Chronic Cluster Headache</th>
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<tr>
<td>A. At least 5 attacks fulfilling criteria B-D</td>
<td>Attacks fulfilling criteria A-E for cluster headache</td>
<td>Attacks fulfilling criteria A-E for cluster headache</td>
</tr>
<tr>
<td>B. Severe or very severe unilateral orbital, supraorbital and/or temporal pain lasting 15-180 minutes if untreated</td>
<td>At least two cluster periods lasting 7-365 days and separated by pain-free remission periods of ≥ 1 month</td>
<td>Attacks recur over &gt; 1 year without remission periods or with remission periods lasting &lt; 1 month</td>
</tr>
<tr>
<td>C. Headache is accompanied by at least one of the following:</td>
<td></td>
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<tr>
<td>1. ipsilateral, conjunctival injection and/or lacrimation.</td>
<td></td>
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<tr>
<td>2. ipsilateral nasal congestion and/or rhinorrhea</td>
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<td>3. ipsilateral eyelid edema</td>
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<td></td>
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<td>4. ipsilateral forehead and facial sweating</td>
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<td>5. ipsilateral miosis and ptosis</td>
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<td></td>
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<tr>
<td>6. a sense of restlessness or agitation</td>
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<tr>
<td>D. Attacks have a frequency from one every other day to 8 per day</td>
<td></td>
<td></td>
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<tr>
<td>E. Not attributed to another disorder</td>
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Table 1 The International Classification of Headache Disorders: Cluster Headache
which time of the year the cluster periods start. This typical rhythmicity of headache attacks and cluster periods has been attributed to a dysfunction of hypothalamus and the biological clock, which will be discussed below.

### 1.2.2 Epidemiology:

The prevalence for CH is reported to be between 0.06% and 0.4% in an adult population (7, 8). In a survey of 18-year old Swedish men, a prevalence of 0.09% was found (9), and in a recent population based Swedish study of twins, aged 40 to 64 years old, the prevalence of CH was 151 per 100,000 (10).

CH is a condition affecting mostly men with females representing 10-30% of the patients. The male predominance is most prominent during the 30’s and after 50 years of age the incidence is the same for men and women (11). The age at onset of CH is usually between 20 and 30 years and CH is very rare in children.

There are several reports showing that the majority of CH patients are heavy smokers (12). It has been speculated why subjects with CH are prone to smoking, as for instance if there would be a genetic predisposition for nicotinic use and CH, or if smoking as such would be a risk factor for catching CH (13).

### 1.2.3 Treatment

The medical treatment in CH includes both acute and prophylactic therapies. Because of the rapid onset and intensity of the headache attacks, a fast-acting therapy is needed. In most CH patients sumatriptan subcutaneously is the first drug of choice (14) but a rapid-acting triptan nasal spray could be an alternative. 100% oxygen is also widely used as headache abortive treatment. Oxygen has no side-effects and could safely be used in patients with cardiovascular diseases. Prophylactic therapy is of importance in CH since many patients have several attacks per day, for several weeks to months. The first drug of choice is verapamil, a calcium-channel blocker that is generally well-tolerated (15). Other alternatives include corticosteroids and lithium carbonate (16, 17). In patients with chronic cluster headache, refractory to medical therapy, deep brain stimulation of the ipsilateral posterior hypothalamus may be considered (18).
1.2.4 Cluster headache pathophysiology

The etiology of CH is still unclear and it is not possible to present one, common pathogenetic model for all features. There are some major aspects that have to be addressed: the trigeminal distribution of the pain, the ipsilateral autonomic symptoms and the episodic pattern.

The trigemino-vascular system

Cerebral vessels are sensory innervated by the trigeminal nerve (19) and distension of the intracranial segments of the internal carotid artery and the proximal parts of the anterior and middle cerebral arteries causes referred pain, in and around the ipsilateral eye. During CH attacks, there are several indications of dilation of major intracranial vessels, such as the ophthalmic and the middle cerebral arteries (20-22). However, vasodilation of cranial arteries is seen in experimental induced pain as well, which indicates that the pain drives the vasodilation and not vice versa (23, 24). The primary source of the pain in CH is still unclear but factors such as lowering of pain thresholds by sensitization of pain receptors may contribute (25). Calcitonin-gene-related-peptide (CGRP) and VIP concentrations are shown to increase in the external jugular vein blood during spontaneous attacks (26) while CGRP is shown to increase during nitroglycerine provoked attacks (27). CGRP is a potent vasoactive peptide, which is found in the trigeminal system, and contributes probably to the vasodilation by an antidromic release from trigeminal afferents (28). VIP is the marker for activation of the parasympathetic nervous system and has vasodilator properties (29).

The trigemino-parasympathetic reflex

The afferent limb of the trigemino-parasympathetic reflex consists of trigeminal nociceptive neurons that supply the face as well as the intracranial arteries. Trigeminal nociceptive output activates parasympathetic fibres in the superior salivatory nucleus in the pons. The efferent limb of the reflex consists of parasympathetic fibres, which travel via the facial/greater superficial petrosal nerves and sphenopalatine ganglion, to supply lacrimal glands and blood vessels in the upper part of the head. There may also be mini-ganglia in the internal carotid canal and in the cavernous sinus, which supply the internal carotid artery with parasympathetic vasodilator fibres (30). The parasympathetic outflow is responsible for some of the autonomic symptoms during CH attacks, such as lacrimation, rhinorrea, nasal congestion and facial flushing but may also contribute to vasodilation of larger intracranial arteries (31).
Hypothalamus and circadian rhythms

The signature feature of CH is the typical rhythmicity of headache attacks and cluster periods which suggests an involvement of hypothalamus and the suprachiasmatic nucleus (SCN), also known as the “biological clock”. The SCN acts as a pacemaker, hereby generating various, endogenous rhythms, for example circadian hormonal secretion. Melatonin, which is synthesized in the pineal gland, has a very strong circadian rhythm generated by the SCN, and serves as a marker for SCN function. In CH, alterations in melatonin secretion are demonstrated in both phases of the disease (32-34). In addition, altered secretion of other hormones regulated by hypothalamus has been reported, especially in active period (32-36), but also in remission (35, 37).

Further evidence of a hypothalamic involvement was apparent when PET studies showed activation of the ipsilateral inferior hypothalamic gray matter during nitroglycerine induced (38) as well as spontaneous attacks (39). In addition, studies with MRI volumetry have shown an increase in grey matter volume, within the inferior posterior hypothalamus (40). PET studies during migraine attacks and experimental induced trigeminal pain (41) has not shown any hypothalamic activation, which indicates that the hypothalamic activity is specific for CH.

In conclusion, the pain and the parasympathetic symptoms during CH attacks may be due to an activation of the trigemino-vascular system and the trigemino-parasympathetic reflex. However, the involvement of a central component, i.e. the hypothalamus in CH pathophysiology is established and CH is nowadays considered as a primary neurovascular headache.

1.3 THE AUTONOMIC NERVOUS SYSTEM -BACKGROUND

The role of the autonomic nervous system is to maintain homeostasis and an optimal environment for cells and tissues by influencing functions such as blood flow, metabolism, growth and sleep-wakefulness. The hypothalamus can be considered as the highest level of integration of autonomic functions. Inputs from the limbic system, brain stem nuclei, subfornical organ and intra- hypothalamic pathways project to the hypothalamic paraventricular nucleus (PVN), which plays a critical role in co-ordination of autonomic, neuroendocrine, and behavioral responses to stress. PVN sends efferents to autonomic regions of the brainstem and the spinal cord and modulates
also stress responses by release of oxytocin/vasopressin, and of hormones from the anterior pituitary, mainly ACTH (42, 43) (figure 1).

1.3.1 The sympathetic nervous system

The cell bodies of the sympathetic, preganglionic neurones are located in the TH 1 to L3 segments of the spinal cord, forming the intermediolateral column. These neurones receive inputs via direct pathways from hypothalamus, autonomic areas in the brainstem as well as from interneurones in the dorsal horn. The preganglionic neurones project to the para-vertebral sympathetic trunk, in which some fibres synapse with cell bodies of postganglionic neurons while others traverse to synapse in small ganglia in the periphery, in close proximity to their target organs.

Sympathetic preganglionic neurons from the upper thoracic segments may synapse in the superior cervical ganglia (SCG). Some of the postganglionic neurons form a
sympathetic plexus around the internal carotid artery and leave via branches of the trigeminal nerve, to supply the eye as well as blood vessels and sweat glands in the medial forehead. The neurons that supply the remaining part of the face project via the external carotid plexus (30). The SCG also sends postganglionic neurons to the pineal gland, regulating the synthesis of melatonin.

Other preganglionic neurons from the upper thoracic segments, as well as neurones from mid-thoracic and lumbar segments, synapse in ganglia of the sympathetic trunk or minor ganglia located in the abdominal and pelvic cavity. The postganglionic axons project to different targets, such as the cardiovascular system, visceral organs, but also to adipose tissue depots. However, some preganglionic neurons synapse directly on functionally postganglionic cells within the adrenal medulla, causing release of adrenaline, and to a lesser extent noradrenaline, into the circulation.

The preganglionic sympathetic neurons use acetylcholine as neurotransmitter, while postganglionic neurons are predominantly noradrenergic. In addition, neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP), and nitric oxide (NO) are co-localized in sympathetic postganglionic neurons, functioning as co-transmitters. Adrenaline is synthesized and stored in the adrenal medulla and released directly into the bloodstream. The adrenal medulla activity is low during basal conditions but increased secretion is recruited in stressful situations to support noradrenaline dependent functions. Both plasma noradrenaline and adrenaline have clear circadian rhythms with nadirs at 02.20 h and 03.20 h, respectively. The adrenaline circadian rhythm is entrained by the SCN, while the noradrenaline rhythm also is influenced by sleep and posture (44). For review see: (45).

In general, increased sympathetic activity accomplishes quick mobilization for emergencies (fight-or-flight). In response to increasing energy demands, metabolic stores are released and circulation is augmented. Enhanced cervical sympathetic activity stimulates pupillary dilation and sweat gland secretion, and increases tonus of eyelid muscles as well as vasoconstrictor tone in the facial skin. Vasomotor tone of major intracranial vessels may also be affected.

A cervical sympathetic deficit may result in miosis and ptosis, decreased sweating and a slight increase in cutaneous facial blood flow, i.e. a Horner’s syndrome (46). But the
vascular regulation of the skin is complex. Thus, during heat and emotional stress increased sympathetic activity triggers cutaneous vasodilation, which results in facial flushing (47).

1.3.2 The parasympathetic nervous system

In general, parasympathetic activity accomplishes restoration, digestion and recovery. Cell bodies of the parasympathetic preganglionic neurons are located in well-defined groups in the brain-stem, forming distinct nuclei. The neurons project via cranial nerves, i.e. the oculomotor nerve (N III) to ocular muscles and glands, the facial nerve (N VII) and the glossopharyngeal nerve (N IX) to lacrimal, nasal and palatal glands and the vagal nerve (N X) to the thoracic and abdominal viscera. There are also cell bodies in the sacral segments of the spinal cord, which project to the lower visceral organs. The parasympathetic preganglionic neurons project their axons to small ganglia just outside or in the wall of the target organs, in which they synapse to postganglionic neurons For review see (45).

Of special interest in cluster headache pathophysiology is the trigemino-parasympathetic reflex (30). Trigeminal nociceptive neurons supply the eyes, mouth, nose and facial skin as well as intracranial arteries and travel to the nucleus salivatorius superior in the brainstem, in which they relay with parasympathetic fibres. Parasympathetic fibres to the upper part of the face leave the brainstem in the facial nerve, join the greater petrosal as well as the Vidian nerves on their way to the sphenopalatine ganglion, for synapse with postganglionic neurons. Via orbital rami and cavernous sinus the parasympathetic fibres project to lacrimal glands and blood vessels in the proximity. There might also be mini-ganglia in the internal carotid canal and in the cavernous sinus, which supply the internal carotid artery with parasympathetic vasodilator fibres. Parasympathetic fibres to the lower part of the face leave the brainstem in the glossopharyngeal nerve and project to the otic ganglion.

Most of the parasympathetic pre-and postganglionic neurons are cholinergic, although co-transmitters such as VIP and NO are of importance.

1.3.3 Autonomic control of the cardiovascular system

The central nervous system is strongly involved in cardiovascular control. The sympathetic tone is generated by widely distributed neuronal groups in the brain and
brainstem, such as the hypothalamic PVN and rostral-ventro-lateral medulla (RVLM), the latter considered to constitute a “vasomotor center”. Efferent neurons project to the intermediolateral column of the spinal cord. Secretions from the adrenal medulla also exert profound cardiovascular influences. The primary location for vagal, preganglionic cardio-inhibitory neurons is the ambiguous nucleus, which is located in the vicinity of the RVLM. The ambiguous nucleus and the RVLM share input from a number of areas and there is a close functional relationship between the sympathetic and vagal systems in order to keep an optimal cardio-vascular balance (48).

1.4 AUTONOMIC DISTURBANCE IN CLUSTER HEADACHE

The facial autonomic symptoms, associated with CH attacks, suggest both a sympathetic as well as a parasympathetic dysfunction. Miosis and ptosis are classical signs of a sympathetic deficit, while lacrimation, conjunctival injection, nasal congestion and secretion indicate a parasympathetic discharge.

1.4.1 Ocular disturbance

Stimulation of the cervical sympathetic nervous system increases pupillary dilation and muscular tonus of the eyelid. Hence, the miosis and slight ptosis in CH are considered to be a result of a sympathetic deficit somewhere along the sympathetic pathway. With pharmacological and physiological pupil function tests, the level of the sympathetic deficit may be detected.

In CH patients, one study with conjunctival instillation of phenylephrine has shown adrenoceptor supersensitivity, indicating an ipsilateral, sympathetic injury, anyway along the sympathetic pathway (49). However, in another study the response to phenylephrine was normal (50). Tests using cocain, tyramine or hydroxy-amphetamine have all demonstrated a diminished pupillary dilation on the symptomatic side, indicating a postganglionic sympathetic injury in CH (50, 51). The trigemino-pupillary response to painless or painful stimulation of the cornea is normally bilateral involving a mydriatic as well as a miotic phase (52). In CH patients in active period, a reduced mydriatic response to painful stimuli was reported on both sides. These findings were interpreted as evidence for a bilateral, presumably central, sympathetic deficit in CH (53).
1.4.2 Lacrimation and vasodilation

CH attacks are often associated with ipsilateral lacrimation, nasal secretion and stuffiness as well as facial flushing and conjunctival injection, of which the three latter are signs of vasodilation. These symptoms have been interpreted as a result of a parasympathetic discharge, i.e activation of the trigemino-parasympathetic reflex (31) (54). Trigeminal stimulation also results in an antidromic release of vasoactive neuropetides, such as CGRP. During CH attacks there is an increase of CGRP into the circulation (26) indicating that vasodilation during CH also is a consequence of the trigeminal activation per se.

1.4.3 Pathological thermoregulatory flushing and sweating

Sympathetic neurons may also mediate vasodilation of the face, since the sympathetic nervous system is responsible for thermoregulatory sweating and flushing. In CH patients thermoregulatory sweating and flushing are diminished on the symptomatic side (55). Paradoxically, there is an ipsilateral increase of sweating and flushing in the medial forehead during CH attacks in patients with ocular deficit, which is greatest in patients with profuse lacrimation (31). A similar pattern is described in patients with a postganglionic sympathetic lesion of other causes (47). This is believed to be due to sprouting of parasympathetic nerve endings into vacated sympathetic pathways causing supersensitivity to cholinergic substances (51).

1.4.4 Salivation

Normally salivation is parasympathetically stimulated and is reduced as a consequence of increased sympathetic activity (“speakers mouth”). Despite the enhanced parasympathetic activity, salivation is decreased on both sides in the mouth during CH attacks (54). This is presumably an effect of a normally increased sympathetic activity in response to pain, and appears to indicate that the site of the sympathetic injury is above the carotid bifurcation.

1.4.5 Cardiovascular control in cluster headache

CH attacks are also associated with various changes of heart rate and blood pressure. Blood pressure is mostly shown to increase during headache attacks (56-58), phenomena that probably are secondary to pain (58).
Most studies of heart rate and rhythm are performed during CH attacks and long-term ECG recordings have shown tachycardia, bradycardia, or other rhythm disturbances (59, 60). In another study, a phase-advance of one hour for the maximum value as well as lower mean heart rate during day time was reported in patients in active period compared to patients in remission and to controls. Interestingly, patients with right-sided pain showed a lower mean heart rate during day-time, compared to patients with left-sided pain and to controls. Moreover, the decrease in heart rate between day and night was smaller in patients with right-sided pain, compared to patients with pain on the left side (61).

Spectral analysis of heart rate variability yields information about the sympathetic-parasympathetic balance and describes the periodical fluctuations of the heart rate due to small adjustments in the control systems (62). During CH attacks, studies of heart rate variability have shown an increase of the high frequency component of the power spectrum associated with a decrease of the low frequency component, a pattern indicating a parasympathetic drive. No changes in heart rate variability during head-up tilt test were found in patients examined between headache attacks (57, 63).

Cardiovascular autonomic function tests have shown inconsistent results. In a recent study, episodic CH patients were examined in and outside a bout, with tests involving both sympathetic and parasympathetic functions. No differences were found between the two illness phases (64). Head-up tilt-test, during active period as well as measurements of muscle nerve sympathetic activity (MSA) during spontaneous an nitroglycerine induced headache attacks, have shown normal responses (58, 65). In contrast, a systemic autonomic impairment involving in particular parasympathetic tests, were seen in both active period and in remission (66).

1.4.6 Catecholamines
Plasma catecholamine concentrations also reflect the sympathetic activity but studies of plasma catecholamine levels in CH patients show conflicting results. Occasional supine day-time noradrenaline levels, as well as response to standing up, have been reported to be normal in CH remission (67). In another study, plasma free and conjugated catecholamine levels were reported to be unchanged during CH attacks, a few hours after an attack as well as in remission (68). This is in contrast to findings in active period, which demonstrate decreased plasma levels of noradrenaline in early morning
as well as in late evening, as an indication of sympathetic hypofunction (69). Furthermore, CH patients in remission as well as in active period have, as an indication of long-term sympathetic function, shown reduced levels of noradrenaline and adrenaline in platelets (70).

In summary, CH attacks are associated with ipsilateral, facial symptoms suggesting a local sympathetic deficit and a parasympathetic discharge. CH attacks are also associated with various changes of heart rate, which may indicate a systemic disturbance of autonomic innervation in CH, although the reports are contradictory. These studies are mainly performed during cluster period or during headache attacks, and the influence of pain and disturbed sleep patterns may be important. However, findings of autonomic dysfunction during CH remission would reflect a mechanism that could be of importance in CH pathophysiology.

The physiological and neurophysiological tests were chosen with a main purpose to investigate cardiovascular functions. However, the autonomic nervous system is involved in many other functions, for instance energy generation and adipose tissue metabolism, which has not been studied in CH previously. The autonomic regulation of adipose tissue metabolism is described below.

1.5 ADIPOSE TISSUE METABOLISM

Adipose tissue represents the largest depot for long-term storage of energy. In a postprandial situation triglycerides (TG) are transported to the adipose tissue and hydrolyzed to free fatty acids (FFA) by adipose tissue lipoproteinlipase (LPL). Insulin stimulates both LPL activity and the uptake of glucose, which in the adipocytes in part is converted to α-glycerophosphate (AGP). AGP forms together with FFA intracellular triglycerides, which are stored in the adipocytes for later requirements of energy (71). Studies have shown that adipose tissue, by breakdown of intracellular glucose, also is a source for lactate production (72, 73). Lactate is transported to the liver and stored as glycogen, a synthesis which is controlled by the glyconeogenetic pathway (figure 2).

Lipolysis is the step-wise breakdown of triglycerides to FFA and glycerol, a reaction that is dependent on hormone sensitive lipase (HSL) activity. FFA:s are to some degree re-utilized by the fat cell for production of new TG (74). This is in contrast to glycerol,
of which the re-utilization is insignificant and for that reason glycerol can be used as a marker of the lipolysis rate (75).

Adipose tissue blood flow (ATBF) is also important for energy storage and mobilization. ATBF increases after food intake, during prolonged exercise as well as during fasting conditions (71). During such circumstances there is a need for transport of substrates to, as well as from, the adipose tissue. Regulation of the ATBF is predominantly under adrenergic influence and adrenaline as well as noradrenaline infusion results in an increase of adipose tissue blood flow (76, 77).

1.5.1  **Hormonal regulation of adipose tissue metabolism**

Catecholamines and insulin are the hormones that acutely have the most pronounced effect on human adipose tissue metabolism. Stimulation of sympathetic neurons in subcutaneous adipose tissue, sympathetic activation during exercise and mental stress and infusion of adrenaline and noradrenaline, into the adipose tissue as well as into the circulation, results in stimulation of lipolysis (77-81). Catecholamines stimulate lipolysis via $\beta$-adrenergic mechanisms (80, 82), partly via noradrenaline released from post-ganglionic sympathetic nerve-endings in the adipose tissue but also via circulating catecholamines released from the adrenal medulla. Among the three $\beta$-adrenoceptors that are functional in human fat cells, the $\beta_1$-and $\beta_2$-adrenoceptors are the most active in lipolysis (83). Activation of the $\beta$-receptors stimulates the adenylate-cyclase system via a stimulatory $G_s$-protein pathway, which results in a step-wise activation of HSL and stimulation of lipolysis (84). Catecholamines, mainly adrenaline, may also have an inhibitory effect on lipolysis via activation of $\alpha_2$-receptors and an inhibitory $G_i$-protein mediated pathway (85).
Insulin is the major anti-lipolytic hormone. Insulin activates insulin receptors situated on the surface of the adipocyte. By activation of a phosphodiesterase mediated pathway, HSL activity is inhibited. In addition, insulin stimulates the re-esterification of FFA to TG within the adipocyte (71).

The role of catecholaminergic activity during basal conditions is somewhat unclear and it seems that other factors could be of importance as well (80). GH is known to stimulate lipolysis, but acts in a slower way than catecholamines (86). A given bolus dose of GH results, after a delay of about two hours, in an increase of lipolysis (86, 87) and a suppression of the nocturnal rise in GH has been shown to influence lipolysis the following morning (88). The effect is probably mostly permissive by improving the sensitivity for catecholamine action (89-91). Cortisol is also believed to modulate adipose tissue metabolism. However, the effect on lipolysis is not completely clarified and a lipolytic as well as an anti-lipolytic effect are reported (90, 92). During the recent years, several other peptides have been discovered as having modulating effects on adipose tissue metabolism. For example natriuretic peptide may be another potent activator of HSL activity and lipolysis (93).
During several years the parasympathetic innervation of adipose tissue was considered to be absent. However, recent studies have shown that systemic nicotine infusion stimulates lipolysis acutely, not only via adrenergic mechanisms but also via activation of local cholinergic receptors interpreting a parasympathetic lipolytic pathway as well (94, 95).

1.6 SOME NEUROENDOCRINE ASPECTS OF INTEREST IN CLUSTER HEADACHE

1.6.1 Growth hormone
GH is secreted from cells in the anterior pituitary and mediates growth and metabolic functions, such as lipolysis. Secretion of GH results in release of intra- and extra hepatic insulin-like growth factor 1 (IGF-1), which is probably responsible for most of the growth-promoting activities of GH. GH has also direct effects on receptors in the periphery, including muscle and fat. During basal conditions, GH levels are low, but due to stimulation GH is secreted in frequent short pulses. In men, most of the daily GH is secreted during early night sleep, but in women GH secretion is more variable over a 24-hour period. For review see (96).

Multiple factors, of which some are shown in fig 3, are involved in the integrated secretion of GH, and the hypothalamic control is of major importance. The predominantly hypothalamic influence on GH secretion is stimulatory, via release of growth hormone releasing hormone (GHRH). The main inhibiting factor is somatostatin, which partly inhibits secretion of GH by direct influence of receptors in the pituitary, partly by inhibition of GHRH release from the hypothalamus.

Various external and metabolic signals modulate GH secretion by influencing hypothalamus and modulation of GHRH and somatostatin neuronal activity. Sleep is one of the most important triggers of GH secretion (97). Other important triggers include acute hypoglycaemia and postprandial glucose decline, exercise and physiological/emotional stress. GH secretion is also lowered with increasing age and body mass (98). During childhood and adolescence, the daily secretion of GH is about 2.0 mg/d compared to 0.02 mg/d in older or in obese adults. Via important negative feedback loops GH and IGF-1 inhibit GH secretion, both at the pituitary and the hypothalamic level.
1.6.2 Hypothalamic-Pituitary-Adrenal-axis

Besides an activation of the autonomic nervous system, stress responses include activation of the hypothalamic-pituitary-adrenal axis (HPA-axis). In fact, stress is the major stimulating factor of the HPA-axis and results in cortisol release from the adrenal cortex. Behavioral/emotional stress signals from the limbic system, as well as physiologic stress signals via brainstem nuclei and the subfornical organ, activate CRH neurons in PVN. CRH neurons project in part to the anterior pituitary, mediating release of adrenocorticotropicin (ACTH). ACTH is secreted in short pulses, stimulating the release of cortisol from the adrenal cortex (fig 3).

The HPA-axis is regulated via two negative feedback loops, in which cortisol acts on hypothalamus and the pituitary, to inhibit the release of CRH and ACTH, respectively. There is also a short-loop feedback in which ACTH acts on hypothalamic CRH neurons as well as an ultra-short feedback loop where ACTH inhibits its own release at the pituitary level.
1.6.3 Insulin

The most important factor for regulation of insulin secretion is glucose. High glucose levels lead to a rapid release of insulin from pancreatic islet cells into the bloodstream. In a state of hypoglycemia, insulin secretion is suppressed. Simultaneously, autonomic nervous activity is stimulated as well as the release of cortisol and GH. It is known that the ventromedial nucleus of hypothalamus contains glucose-sensing neurons that trigger the neuroendocrine responses to hypoglycemia (99). The influence of insulin on adipose tissue metabolism is previously described.

1.6.4 Prolactin

Prolactin is released from the anterior pituitary and is under inhibitory control of dopamine, acting on dopamine₂ receptors. Stimulating factors include stress, sleep, pregnancy and lactation but also neural factors such TRH, estrogen, serotonin, VIP. Prolactin secretion has a pronounced 24-hour rhythm with a maximal nocturnal peak in the early morning hours.

1.6.5 Gonadotropins (LH, FSH) and testosterone

Gonadotropin releasing hormone (GnRH) is released from hypothalamus and stimulates synthesis and secretion of both LH and FSH from the pituitary. LH and FSH acts on the ovaries as well as the testes, stimulating release of sex hormones such as estrogen and testosterone.

1.6.6 Thyrotropin

TRH stimulates the release of thyrotropin (TSH) and prolactin from the anterior pituitary. TSH secretion is inhibited by sensitive feedback mechanisms, partly exerted by peripheral thyroid hormones but also by somatostatin and dopamine.

1.6.7 Melatonin:

The circadian rhythm of melatonin secretion is strongly entrained by the SCN, thus melatonin has been considered as an important marker of SCN activity. The principal environmental stimulus of melatonin production is the light-dark cycle. In response to darkness, projections involving the retino-hypothalamic pathway, SCN and the sympathetic nervous system reach the pineal gland, hereby stimulating release of melatonin via β-adrenergic mechanisms (100). In humans, melatonin levels are low during the day and high during the night with a nocturnal peak around 02.00 h (101).
1.7  HORMONAL FINDINGS IN CLUSTER HEADACHE

Altered hormonal regulation in active cluster period has been reported in a number of studies. There are only few reports of hormonal secretion in the remission phase, some of which have indicated a persistent alteration of secretory patterns.

1.7.1  Growth hormone

The documentation of basal GH secretion in CH is sparse. In one study by Chazot and co-workers (32), plasma GH patterns were abnormal in some patients in active period, showing a bimodal profile attributed to an advance of the GH evening peak. Klimek reported that basal levels of GH in CH patients during active period, between attacks and before any medication, were in the normal range and did not differ from those obtained in controls. Results of dynamic tests with insulin, oral cyproheptadine, oral bromocriptin and intramuscular metoclopramide did not differ between the patients and controls, concluding that GH release was not disturbed in CH (102, 103). Activation of serotonergic receptors is known to stimulate GH secretion. In remission, challenge tests with serotonergic agonists such as quipazine and sumatriptan have shown normal results (104, 105) but in active period the response to sumatriptan was significantly blunted (105).

In addition, to its inhibiting effect on GH release somatostatin has been shown to have central pain-modulatory properties (106) and octreotide, a somatostatin analogue, has been shown to be effective in the acute treatment of CH attacks (107). However, if there are any alterations in basal plasma somatostatin levels in CH patients, is still unclear.

In conclusion, one study of basal GH secretion indicates an altered GH circadian production in active period. Otherwise there are no previous indications of any major disturbances in GH metabolism in CH.

1.7.2  HPA-axis

There are several indications of a disturbed HPA-axis in CH. In active period, studies have shown increased 24-hour production as well as increased basal cortisol morning levels (32-34, 37, 69) and modifications of the cortisol circadian rhythmicity is also demonstrated (32, 34). In remission, there are more varying results and even though
occasional basal cortisol morning levels have been found to be increased (37, 108), 24-hour production was shown to not differ in CH patients compared to controls (34). Dexametason tests have shown ordinary responses in both phases of CH indicating that the feedback mechanisms are normal (108, 109). However, CRH test has revealed blunted cortisol responses, both in active period and in remission, interpreted as an indication of down-regulated ACTH receptors in the pituitary. Insulin tolerance test has revealed blunted ACTH and cortisol responses in both phases of CH, as an indication of defective regulation of hypothalamic neurons that control the HPA-axis (37).

In summary, there are indications of an increased HPA-axis activity in both phases of CH and in active period the cortisol circadian rhythm seems to be disturbed.

1.7.3 Insulin
There are no studies of basal insulin secretion in CH. However, it is possible that insulin related mechanisms are involved in CH pathophysiology. In migraine, which also is considered as a neurovascular headache disorder, impaired insulin sensitivity has been found (110) as well as polymorphisms in the insulin receptor gene, suggesting an involvement of insulin regulation in the pathogenesis of migraine (111). To the best of our knowledge, no such studies are undertaken in CH.

1.7.4 Prolactin
In CH active period, the diurnal rhythm of prolactin has been reported normal (32) or altered (112). Prolactin production over 24 hours has been reported to be normal in active period (112) or to be reduced both in active period and in remission with a blunted nocturnal peak (35). In active period, the response to metoclopramid, a dopamine _2_ - receptor blocker has shown to be decreased (103) whereas the response to bromocriptin, a dopamine agonist has revealed normal suppression (102). TRH test has shown normal (113) as well as blunted responses, however, the latter only shown in women (35). Studies of serotonergic regulation of prolactin has revealed indications of a 5HT1A-receptor hypersensitivity during cluster periods (114) but normal responses to cyproheptadin and quipazine indicating normal functions of 5-HT2C (102) and 5-HT3 receptors (104).
In summary, there are indications of altered prolactin regulation in CH, both in active period and in remission. This might be compatible with dopaminergic hyperactivity and a down-regulation of prolactin producing cells in the pituitary.

1.7.5 Gonadotropins- LH and FSH

In a number of studies, serum levels of LH and FSH have been reported normal, both during and between the active cluster period (35, 102, 104). However, there are reports of reduced LH levels and increased FSH levels in active period (115) and altered LH pulsatility over 24 hours is also reported (116). Challenge with LHRH has revealed reduced as well as normal LH responses and increased as well as normal FSH responses in active period or in chronic cases (102, 115).

In summary, studies of LH and FSH secretions have shown contradictory results in CH and no further conclusions could be drawn.

1.7.6 Testosterone

During cluster periods or during headache attacks, several studies have reported reduced morning testosterone levels (115, 117, 118). Reduced 24-hour testosterone production, an altered circadian rhythm, and reduced minimum and maximum values (35, 36) are also reported. In remission, serum testosterone levels and secretory patterns are demonstrated to be normal (35). Reduced testosterone levels have been interpreted as a result of pain and stress during active period.

1.7.7 Thyrotropin

There are some studies of the TRH-TSH axis in CH. Basal TSH, T3 and T4 levels is reported normal in both illness phases (35, 113). In active period, TSH response to TRH stimulation has been found to be reduced (109) however, Waldenlind and co-workers did only demonstrate this finding in women (35).

1.7.8 Melatonin

In CH active period, reduced 24-hour plasma levels of melatonin, and reduction as well as phase-shifts of the night time peak are reported (32-34, 109). In addition, 12-month mean levels of urinary melatonin has been reported to be reduced in CH patients in remission and in active period compared to controls, but no clear difference could be shown between patients in active period and in remission (119). Significantly lowered
excretion of urinary 6-sulphatoxymelatonin has also been reported, both in active period and in remission (120).

In summary, melatonin is an important marker for hypothalamic activity and circadian rhythms. In CH period, there are several indications of an abnormal melatonin secretion which together with reduced urinary melatonin excretion in remission suggest a permanent hypothalamic disturbance.
2 AIMS
The overall objective with this project was to focus on a better understanding of autonomic and neuroendocrine functions in CH. As a metabolic marker of systemic autonomic activity, adipose tissue lipolysis was studied, a metabolic function that in part is regulated by hypothalamus.

Paper I. The aim of the study was to investigate if CH patients showed altered nocturnal lipolysis, as a sign of disturbed nocturnal sympathetic function. Interstitial glycerol concentrations, the end-product of adipose tissue lipolysis, were compared between CH patients in remission and in active cluster period, but between headache attacks. The results in remission and cluster period were also compared with healthy controls.

Paper II. The majority of the CH patients are habitual smokers. The aim of the study was to investigate to which extent smoking habits affect nocturnal lipolysis. Nocturnal lipolysis was studied in habitual smokers during short-term tobacco withdrawal. The results were compared to a control group of non-smokers.

Paper III. The lipolytic activity may be affected by disturbed β-receptor function. The aim of the study was to investigate the lipolytic response to NA in CH patients in remission, as an estimate of the local β-receptor function in adipose tissue. Lipolysis rate was compared between CH patients and healthy subjects.

Paper IV. The aim of the study was to investigate the nocturnal secretion of noradrenaline, GH, cortisol and insulin, hormones with potential effect on lipolysis and of which the secretion at least partly is regulated by hypothalamic activity. Hormone levels were measured in venous blood and the results were compared between CH patients in remission and healthy subjects.
3 MATERIALS AND METHODS

3.1 SUBJECTS
Consecutive ambulatory patients from the Department of Neurology at Söder Hospital as well as Karolinska University Hospital, Huddinge in Stockholm, Sweden were recruited to these studies. All patients suffered from CH according to International Headache Society criteria (1). Healthy subjects were members of the hospital staff or enrolled by advertising in the newspaper. They were all healthy and did not suffer from headache. None of the subjects was on long-term medication. The healthy subjects matched the CH group with regard to sex, age and BMI (study I) or with regard to sex, BMI and smoking habits (study III, IV).

All studies have been ethically approved and informed consent was obtained from all patients and healthy subjects.

3.1.1 Comments study I
Of the ten CH patients included in this study, nine patients had episodic CH. Six were studied both in active period and in remission, while three were studied in remission only. One patient had chronic CH. Of the patients that were studied during active headache period, no one had headache attacks during the study night. Two CH patients suffered from chronic bronchitis of which one medicated with glucocorticoid inhalations and the other one with acetylcystein and hypnotics (zolpidem). These drugs were not taken during the experiments. None of the episodic CH patients had taken prophylactic treatment. The patient with chronic CH had taken lithium for one month but stopped the medication six days prior to the start of the study.

Fifteen healthy, headache free volunteers were enrolled, nine smokers and six non-smokers. The healthy subjects matched the CH group with regard to age and BMI.

3.1.2 Comments study II
Sixteen male, healthy volunteers were recruited, nine smokers and seven non-smokers. For lipolysis studies, nine smokers and six non-smokers participated. Adipose tissue blood flow was analyzed in six smokers and in seven non-smokers. In the smoking group, each individual smoked at least 10 cigarettes per day. They were all habitual
smokers since 10 years or more. Smokers and non-smokers were matched with regard to sex, age and body mass index (BMI).

3.1.3 Comments study III+IV
Ten CH patients participated in study III and nine in study IV. Of the 19 CH patients enrolled, six participated in both studies. All CH patients were in remission phase. One CH patient had mild hypertension and medicated with hydrochlorotiazid +amilorid (study III + IV) and one had allergy which was treated with a histamine-1 antagonist and nasal glucocorticoids (study IV). Otherwise all patients were healthy and did not take any chronic medication. In addition, a total of 20 healthy volunteers were recruited, of which seven participated in both studies. CH patients were significantly older than healthy subjects. For demographic data see paper III and IV.

3.2 METHODS

3.2.1 Study I-III

Microdialysis device
The principle of microdialysis is to mimic the function of a blood vessel. The microdialysis catheter (CMA 60, 0.6 x 30 mm, CMA/ Microdialysis AB, Stockholm, Sweden) consists of a double-lumen cannula with a semi-permeable membrane at the top (molecular cut-off 20.000 kDa). The catheter is inserted into the adipose tissue with help of a guide cannula (1.4 x 54 mm), and the inlet of the catheter is connected to a high precision pump (CMA 106 or 107; CMA/ Microdialysis AB, Stockholm, Sweden). When continuously perfusated with a physiological solvent (CMA Perfusion fluid T1; Na+ 147 mM, K+ 4 mM, Ca2+ 2.3 mM, Cl− 156 mM, PH 6, osmolality 290 mosm/kg; CMA/ Microdialysis AB, Stockholm, Sweden), there will be a diffusion of substances back and fourth over the dialysis membrane. The outgoing dialysate mirrors the composition of the extracellular space. It is also possible to add different substances to the perfusion fluid which gives an opportunity to mimic the release of substances in situ, and to measure the acute metabolic response (121-123).

Variations in adipose tissue blood flow (ATBF) may influence extra-cellular fluid composition. In the present study, ATBF was measured by ethanol technique (124). When adding ethanol (5 mM) to the perfusion fluid (Ringer solution), there will be a diffusion of ethanol through the dialysis membrane. The ratio between out-going and
in-going ethanol mirrors changes in adipose tissue microcirculation. A high ratio indicates a low blood flow and vice versa.

**Analyses**

Dialysate concentrations of glycerol, glucose and lactate were measured with a commercial method using enzymatic reagents and colorimetric measurements (CMA 600 Microdialysis Analyser; CMA/ Microdialysis AB, Stockholm, Sweden). The ethanol ratio, used for flow measurements was determined using an enzymatic, fluorometric method (125).

**Microdialysis**

*Comments on study I and II*

Basal subcutaneous glycerol levels were measured at night from two catheters in each subject, since we occasionally have had experienced technical problems with poor recovery from some of the microdialysis probes. After analysis of catheter function one of the catheters was selected for further evaluation, as described below.

The microdialysis catheters were inserted into the abdominal subcutaneous adipose tissue, on each side of the umbilicus and perfusated with a physiological solution at a
rate of 0.3 μl/min. Dialysates were collected at hourly intervals (CH patients) or 30 minutes intervals (healthy subjects) from midnight until 06.00 hours. The fractions were kept in -20°C for later analysis of glycerol, glucose and lactate. In 13 of the healthy controls, a third catheter for simultaneous blood flow measurements was implanted about five centimeters away from the second one and perfusated with Ringer solution containing 5 mM ethanol at a rate of 1 μl/min. Dialysate for blood flow measurements were collected in hourly intervals and kept in refrigerator for 48 hours at the most, before analysis.

During the experiment, study subjects were only allowed to drink water and they were not allowed to smoke. However, three of the CH patients in active period and one in remission had an evening snack of maximum 300 kcal at 18.45 - 22.45 hours. Neither the microdialysis pattern nor absolute glucose or glycerol levels were affected by the snacks (data not shown). The smokers were refrained from smoking for at least seven hours prior to the test.

**Evaluation study I and II**

After analysis of all samples, one of the two catheters was selected systematically in accordance to criteria that were predetermined considering missing values and recovery. First, the majority of the samples had to have dialysate glucose concentrations of 3.5 mmol/L or more, as a measure of recovery, otherwise the catheter was annulled. If both catheters fulfilled the first criteria, the catheter with the least number of missing values was picked.

The data of glycerol, glucose and lactate were analyzed considering concentrations (study I and II), as well as temporal patterns (study I). The night was divided into two-hour intervals, i.e. 24.00 - 02.00 hours, 02.00 - 04.00 hours and 04.00 - 06.00 hours, (study I) or in three-hour intervals i.e. 24.00-03.00 h and 03.00-06.00 h (study II). In study I, the mean concentrations for each interval were compared between the two groups of CH patients and healthy controls as well as pair-wise between remission and cluster period, in the patients studied twice. In study II, the mean concentrations for each interval were compared between smokers and non-smokers. The nocturnal temporal pattern was evaluated in two ways. First, the up- or downshift trends throughout the night were studied for CH patients in active period, patients in remission
and healthy controls separately. The mean concentration for the time intervals were stepwise compared. The differences in the time course of the curves between the study groups were assessed by calculating the difference (Δ) in concentration between the first and the second interval, as well as between the second and third interval. Subsequently, Δ-values were compared between patients in cluster period, patients in remission and healthy controls.

Comments on study III
All subjects were fasting, although they were allowed to drink water. They were not allowed to smoke. Two microdialysis catheters were inserted into the abdominal subcutaneous adipose tissue, on each side of the umbilicus and perfused with Ringer solution at a rate of 0.3 μl/min. In addition, a third catheter for simultaneous blood flow measurements was implanted about five centimeters away from the second one and perfused with Ringer solution containing 5 mM ethanol, 1 μl/min. From the two first catheters, dialysates were collected every 20 minutes for two hours after which the catheters were perfused with noradrenaline (10^{-6}M) (for further information see paper III). Dialysates were collected at 20 minutes intervals for another two hours. The fractions were kept in -20°C for later analysis of glycerol and glucose. In the catheter used for blood flow measurements, dialysates were collected every 20 minutes during the whole study period and kept in refrigerator for 48 hours at the most, before analysis.

Evaluation study III
After analysis of all samples, one of the two catheters was selected systematically in accordance to the same criteria reported above. The mean basal level for glycerol and glucose was calculated from three samples collected during 60 minutes before addition of noradrenaline. Glycerol data are presented as the maximum increase as well as the average increase during infusion of NA and expressed as a percentage of basal level as well as in absolute values when applicable. Adipose tissue blood flow is presented as ethanol outflow/inflow ratio.

3.2.2 Study IV
Assays
All samples were analyzed at the Laboratory of Clinical Chemistry at Karolinska University Hospital, Huddinge with accredited, commercial methods. The analyses
were carried out at two time points. Serum GH was determined by FluorolmmunoAssay (Wallac Oy, Finland) with a sensitivity of 0.01 μg/L. The intra-assay coefficients of variation (CV%) were 4.00% and 3.55% (time point 1 and 2), respectively and the inter-assays CV% were 2.77% and 1.86%. Serum insulin and serum cortisol were determined by an immunometric method (ECLIA) (Roche Diagnostics Gmb H, Mannheim, Germany). For insulin the sensitivity for the assay was 1.39 pM. Intra-assays CV% were 3.46% and 2.15% respectively and inter-assays CV% were 2.33% and 1.75%. For cortisol the sensitivity was 1.00 nM and intra- and inter-assays CV% were 2.87% ; 3.44% and 1.82% ; 3.02%, respectively. Plasma noradrenaline was determined by a HPLC-system with a sensitivity of 0.3 nM and intra- and inter-assay variations were 4.6% ; 6% and 3.8% ; 5%, respectively.

Comments on study IV
All subjects were fasting from 18:00 hours, but they were allowed to drink water. They were not allowed to smoke. Subjects arrived to the hospital at 20:30 hours and stayed over night. At 21:00 hours, a cannula was inserted into an antecubital vein. Subjects went to bed at 23:00 hours and during the night there was a continuous dim light in the room. Venous blood samples were collected between 23.00 and 07.00 hours, at hourly intervals for noradrenaline, cortisol and insulin and at thirty minute intervals for GH. Blood samples for GH, cortisol and insulin were centrifuged and serum was stored at -20°C until analysis. Blood samples for analysis of noradrenaline were collected in heparinized ice chilled tubes, centrifuged immediately at 4°C and plasma was stored in -70°C.

Evaluation study IV
The hormonal secretion were evaluated considering nocturnal concentrations, temporal pattern, maximum /minimum values as well as time to maximum /minimum values, when applicable. The results were compared between CH patients and healthy subjects.

3.3 STATISTICS
In the case of normally distributed data, Students t-test for independent samples was used for comparisons between two different groups. When data were estimated not to be normally distributed, Mann-Whitney U test or Wilcoxon’s test for matched pairs was applied. In study IV, data were analyzed using ANOVA for repeated measurements design. Time (9 or 16 time points between 23 and 07 hours) and group
(CH and controls) were included as dependent and independent factors in the model. BMI was included as a covariate for the GH and insulin analyses and age for NA analysis. Interaction effect was tested for the time x group factor. Presence of interaction effect reveals that change within groups is not similar over time. Since the GH values were not normally distributed they were log(e) transformed. Time to the maximum or minimum value was analyzed by use of Mann-Whitney U-test. A p-value of < 0.05 was considered to be statistically significant.
4 RESULTS

4.1 STUDY I AND II

4.1.1 Adipose tissue glycerol

Study I: The nocturnal glycerol concentrations for CH patients in active period, in remission and for healthy controls are presented in fig 5. The average glycerol level for the whole investigation period, presented as median and interquartile range (IQR) was 255 (211;319) μmol/L for the control group. In CH patients in active period the average level was lower, 69% of the control group level (Mann-Whitney U test, p<0.05). In CH patients in remission, the average nocturnal glycerol concentration was 81% of the control group level, but this difference was not significant. Compared to controls, CH patients in active period had significantly lower glycerol concentrations during all three two-hour intervals. For CH patients in remission, the glycerol concentrations were significantly lower at 02.00 - 04.00 hours and 04.00 - 06.00 hours. Moreover, CH patients in remission had a significantly different nocturnal lipolysis rhythm compared to healthy subjects, which was shown by decreasing glycerol concentrations between the first (24.00 – 02.00 hours) and the second (02.00 - 04:00 hours) interval. In healthy
subjects, lipolysis increased significantly during the night. In the six CH patients studied twice, there were no significant differences in glycerol levels or in lipolysis rhythm, between active period and remission.

To investigate if habitual smoking influenced nocturnal lipolysis, the aim of study II was to compare nocturnal lipolysis between smokers and non-smokers during short-time tobacco withdrawal. The average nocturnal glycerol level was 247 (224 ; 312) μmol/L (median, first and third quartile), for smokers. In non-smokers the glycerol level was slightly higher, 292 (205 ; 382) μmol/L, but this difference was not statistically significant. Moreover, comparing glycerol concentrations for the three-hour intervals i.e. 24.00-03.00 and 03.00-06.00, no significant differences between smokers and non-smokers were found.

4.1.2 Adipose tissue glucose and lactate
In CH patients (study I), nocturnal adipose tissue concentrations of glucose and lactate were within physiological ranges and there were no significant differences between the two CH groups or when comparing either patient group with healthy subjects.

Comparing smokers and non-smokers (study II), there were no significant differences in interstitial glucose levels, neither when considering the average nocturnal glucose concentrations, nor when evaluating the three-hour intervals. The average nocturnal lactate concentration during the night was 0.96 (0.83; 1.04) mmol/L (medians, first and third quartiles) in smokers and 1.34 (1.19;1.48) mmol/L in non-smokers. This trend, towards lower average nocturnal lactate level in smokers, was not statistically significant (Mann-Whitney U test, p=0.059) but when the night was divided into the three-hour intervals, it was shown that smokers had significantly lower lactate concentrations between 03:00 and 06:00 hours.

4.1.3 Adipose tissue blood flow
ATBF was only measured in healthy subjects. In smokers and non-smokers together (study I), the ethanol outflow/inflow ratio was constant throughout the night (mean ratio 0.23 ±0.18) and there were no significant differences between smokers and non-smokers analyzing the two hour intervals. When smokers and non-smokers were compared separately (study II), the average nocturnal ethanol outflow/inflow ratio was 0.15 (0.05; 0.21) (median, first and third quartiles) for smokers and 0.17 (0.09; 0.40) for
non-smokers. Both smokers and non-smokers demonstrated constant ratios throughout the night and there were no significant differences comparing the three-hour intervals.

When pooling data for smokers and non-smokers (*study I and II*), there was no correlation between ethanol outflow/inflow ratio and dialysate glycerol concentrations. However, when calculating the correlation between ethanol outflow/inflow ratio and glucose there was a significant negative correlation, $r = -0.67$.

In summary, *study I and II* showed diminished nocturnal lipolysis in CH patients, both in active period and in remission. In addition to reduced lipolysis, CH patients in remission showed an altered temporal pattern of the nocturnal lipolysis rhythmicity. Within a group of healthy subjects, there were no differences in nocturnal lipolysis between smokers and non-smokers. Interstitial glucose and lactate concentrations did not differ between CH patients and controls neither did the glucose concentrations differ between smoker and non-smokers.

### 4.2 STUDY III

#### 4.2.1 Adipose tissue glycerol

Noradrenaline was infused into the adipose tissue and the increase in glycerol concentrations was considered as the $\beta$-receptor response. Addition of noradrenaline to the perfusion fluid resulted in a distinct elevation of adipose tissue glycerol levels, both in CH patients and in healthy subjects. Noradrenaline was infused during 120 minutes. During this time the average increase in glycerol, expressed as percentage of basal level, was $121 \pm 49 \%$ (mean $\pm$ SD) in CH patients compared to $77 \pm 41 \%$ in healthy subjects, a difference that was statistically significant ($p<0.05$). The maximum increase after infusion of noradrenaline was higher in CH patients ($195 \pm 83 \%$) than in healthy subjects ($135 \pm 60 \%$), however, the difference was not significant ($p=0.08$). In all CH patients except one, the maximum concentration after addition of NA occurred in the first 20 minute sample. In healthy subjects, the peak concentration occurred in the first 20 minute sample in five subjects, in the second in four subjects and in the third in one subject. However, there was no statistically significant difference in time for the max peak concentration between CH patients and healthy subjects. Baseline levels for interstitial glycerol were similar in CH patients and healthy subjects (see paper III, table 2).
CH patients were found to be as a mean 10 years older than healthy subjects. For that reason, a regression analysis was carried out to evaluate if age influenced the outcome of the variables maximum glycerol increase and average glycerol increase in the age range of CH patients and controls. However, no significant correlation was found.

4.2.2 Adipose tissue blood flow:
It was possible to measure ethanol outflow/inflow ratio in eight CH patients and six healthy subjects. Basal ratios did not differ between CH patients and healthy subjects nor did the average ethanol ratios after addition of noradrenaline to the adjacent catheter (data not shown in the paper). In healthy subjects, ethanol ratio dropped 11% in the first twenty minute sample after addition of noradrenaline, compared to 1% in CH patients, but there was no significant difference between the groups.

In summary, in response to noradrenaline infusion the average glycerol increase was significantly higher in CH patients than in healthy subjects. There were no differences in ATBF between the groups. The results indicate an increased β-receptor response to noradrenaline in CH patients in remission.

4.3 STUDY IV

In order to investigate the nocturnal secretion of hormones with potential effect on lipolysis, nocturnal levels of NA, GH, cortisol and insulin were measured in venous blood. The nocturnal profiles for GH are shown for CH patients and healthy controls in fig 6.

4.3.1 Growth hormone
GH varied significantly over time in both groups but the nocturnal pattern was different in CH patients compared to healthy subjects. This was shown statistically by the interaction effect between time and group, and clinically observed as a smaller increase between 24:00 and 01:00 hours in CH patients, reaching a level of 0.41 (0.22; 2.14) µg/L (median and IQR), compared to 2.83 (1.93; 3.90) in controls. The maximum GH peak values did not differ significantly between the groups, neither did the mean nocturnal GH concentrations. Time to the maximum peak did not differ significantly between the groups, but in two CH patients the maximum GH peak occurred as early as
23:00 hours. In six CH patients, the max peak occurred between 24:00 and 02:30 hours and in one at 05:00 hours. In healthy controls, the maximum peak occurred between 24:00 and 02:30 hours in eight subjects and between 06:00 and 07:00 hours in two subjects.

4.3.2 Noradrenaline:
There were no significant time-related changes in noradrenaline concentrations over the night, and the nocturnal temporal patterns did not differ between CH patients and healthy subjects. Nocturnal noradrenaline concentrations did not differ between CH patients and controls, neither did the nocturnal minimum noradrenaline level or the time to the nadir.

4.3.3 Cortisol:
Cortisol concentrations increased significantly during the night but there was no difference in the temporal patterns between the groups. The nocturnal cortisol
concentrations, the levels of the maximum cortisol peaks, the minimum levels, and the
time to the maximum and minimum levels, respectively did not differ either.

4.3.4 Insulin:
There were no significant time related changes in insulin concentrations during the
night and the temporal patterns did not differ between the groups. Neither, did the
nocturnal insulin concentrations during the night.

In summary, the results indicate an altered nocturnal GH secretion in CH patients in
remission. No differences were observed in noradrenaline, cortisol or insulin secretions
between patients and controls.
5 DISCUSSION

The present thesis focuses on the autonomic as well as the hypothalamic disturbance in CH. The primary objective was to evaluate the likelihood for a systemic autonomic disturbance, which possibly also could be of a central origin. The facial sympathetic deficit in CH is demonstrated to be an effect of a peripheral disturbance (126), while studies of heart rate and heart rate variability indicate a systemic, sympathetic-parasympathetic imbalance of a possible central origin (57, 60, 63). Since most of the latter studies are performed during cluster period only, the influence of pain and disturbed sleep patterns has to be considered. If changes of autonomic functions in the remission phase can be demonstrated, they would support a permanent autonomic disturbance, which might be involved in CH pathophysiology.

In this project we wanted to introduce a new marker for the study of systemic autonomic nervous system activity in CH. Our first intention was to study autonomic tone before, during and after cluster headache attacks. To be able to study spontaneous attacks, there was a demand of a marker that could be measured continuously, and for a long time, such as adipose tissue metabolism. The relationship between sympathetic nervous system activity and adipose tissue lipolysis is described in a number of studies (78-80). There is also a relationship between the adipose tissue and hypothalamus, illustrated by the release of leptin, an adipose tissue hormone which acts on receptors in the hypothalamic arcuate nuclei, thereby decreasing food intake (127, 128). Moreover, there are some indications of a direct relationship between leptin and the sympathetic nervous system, at least in animals. For these reasons we decided to study lipolysis as a metabolic marker of systemic sympathetic activity in CH.

5.1 METHODOLOGICAL CONSIDERATIONS

5.1.1 Microdialysis

It is an advantage to use a method which makes it possible to study lipolysis in situ. With microdialysis technique, it is possible to continuously measure glycerol concentrations in subcutaneous adipose tissue, as an estimate of the lipolytic rate (121, 122, 129). This method allows small sampling volumes and long-term continuous monitoring with a minimum disturbance of night rest. With the study subjects asleep,
study conditions will be standardized and reproducible. It is also possible to add different substances to the perfusion fluid, which gives a unique opportunity to mimic the release of substances in situ, and to measure the acute metabolic response.

The microdialysis technique also has some disadvantages. When implanting the probe there may be a bleeding which sometimes causes blood clots onto the dialysis membrane, resulting in diminished diffusion volumes. To be able to mirror the composition of the extra cellular space, the flow rate through the microdialysis catheters has to be low, which may result in small sampling volumes sometimes not sufficient for analyzing. For these reasons we always inserted two microdialysis catheters in each patient, even though only one was selected for further analyses.

In our experience the sampling intervals have to be at least 15 to 20 minutes, an interval that may be too long to detect rapid metabolic changes. In fact, it appeared to be difficult to detect the fast changes in glycerol concentrations during headache attacks, thus we decided to focus on the headache free periods.

When designing a microdialysis study, it is important to consider the time interval between insertion of the probes and collection of the samples. Implanting the probe causes transient damage to the tissue and a certain time for the metabolites to reach a steady state situation is probably needed. In one study, ATP and glucose levels were found to be transiently increased during the first 15 minutes after insertion, which indicated an equilibration period of 30-45 minutes to be sufficient (130).

Variations in interstitial glycerol concentration could be a result of an altered metabolism but could also reflect physiological changes in microcirculation or even artifacts. An increase in adipose tissue blood-flow (ATBF), expressed as a decreased ethanol outflow/inflow ratio, results in diminished interstitial glycerol concentrations, as circulating glycerol is transported out of the adipose tissue(131). In contrast, interstitial glucose levels will be increased (132). A lack of correlation between ethanol outflow/inflow ratios and glycerol concentrations would indicate true variations in glycerol levels and not changes in ATBF. On the other hand, an inverse correlation between ethanol outflow/inflow ratio and glucose would indicate the validity of the ethanol method. It is previously shown that interstitial glucose concentrations correlate well with glucose levels in plasma (133). Thus, stable interstitial glucose
concentrations, within the physiologic range, are also an indication of the reliability of
the method. In study I-III we could not demonstrate any major differences in ethanol
outflow/inflow ratios or interstitial glucose concentrations, between CH patients and
healthy subjects. No significant correlations were found between glycerol
concentrations and ATBF but in contrast, significant inverse correlations were found
between ATBF and glucose concentrations (study I-II). In summary this indicates that
our findings represent true variations in glycerol concentrations and not changes due to
microcirculation or artifacts.

5.1.2 Venous blood tests:
All samples were analyzed at the Laboratory of Clinical Chemistry at Karolinska
University Hospital, Huddinge with accredited, commercial methods. Noradrenaline,
cortisol and insulin were measured in hourly intervals, an interval that would reflect the
rather slow nocturnal variations of these hormones. GH is secreted in short bursts. For
that reason we measured GH concentrations every thirty minute during the entire night.
This interval was considered short enough to reflect a major part of the GH secretion.
Shorter sampling intervals were considered not to be possible since that would have
disturbed the subjects night sleep too much. All samples were collected during the night
and there was a continuous dim light in the room. It cannot be excluded that the light,
together with a new milieu, may interfere with sleep. However, this is a problem that is
shared with all similar studies and the most important way to reduce variations between
study groups is to standardize the procedures as much as possible.

5.2 DISCUSSION OF RESULTS OF STUDY I-IV

5.2.1 Study I
In study I, we used microdialysis technique to study nocturnal lipolysis, as an estimate
of sympathetic activity in cluster headache patients in active period, in remission and
healthy subjects. In this study, we could demonstrate that CH patients, irrespective of
illness phase, had diminished nocturnal lipolysis. In addition, patients in remission had
a different nocturnal lipolysis rhythm.

The sympathetic nervous system stimulates lipolysis via activation of β-receptors in the
adipose tissue. This is predominantly effectuated via release of noradrenaline from
sympathetic nerve-endings but also via catecholamines in the circulation (77, 80, 82,
The present study is the first study that has been able to demonstrate systemic, metabolic changes in CH. Assuming that nocturnal lipolysis reflects the general sympathetic tone, it may be interpreted that the sympathetic activation of lipolysis is diminished in CH, irrespective of illness phase. Whether this disturbance is localized in the hypothalamus or somewhere downstream along the sympathetic pathway is not possible to establish with this study design.

Another interesting finding was the altered temporal pattern of nocturnal lipolysis in CH patients in remission. In contrast to healthy subjects, who in accordance with previously studies showed a distinct nocturnal rhythm with a gradual increase during the night (129), CH patients demonstrated declining glycerol concentrations between 24.00 and 04.00 hours. Also the sympathetic nervous system shows a circadian rhythm, which is reflected of nadirs in the middle of the night for adrenaline and noradrenaline secretion (44). Hypothalamus i.e the suprachiasmatic nucleus, is responsible for the generation of various endogenous rhythms. Hypothalamus is also an important area for regulation of the autonomic nervous system. Thus, the finding of an altered temporal pattern for lipolysis may indicate a hypothalamic involvement in CH and a central cause for the systemic autonomic disturbance.

Besides sympathetic activity, other factors may influence lipolysis. In contrast to catecholamines, insulin has a potent anti-lipolytic effect.(122, 135). Growth hormone also stimulates lipolysis (86, 89), but the effect of cortisol on adipose tissue metabolism is still controversial (90, 92). Diminished lipolysis could be a result of reduced hormonal secretion, but a peripheral disturbance in the adipose tissue, such as altered β-receptor or insulin receptor sensitivity are other possibilities. Environmental factors, such as tobacco smoking, may influence lipolysis as well.

In conclusion, we found altered lipolysis in patients with CH, both in symptomatic periods and in remission. The altered lipolysis may be due to a reduced nocturnal sympathetic activity but also to a hypothalamic disturbance and may indicate a central sympathetic dysregulation. However, other causes of diminished lipolysis have to be further evaluated.
5.2.2 Study II

Nicotine influences several metabolic events, such as lipid metabolism, at least acutely (136). There are several reports showing that the majority of CH patients are heavy smokers (12) thus, the high number of smokers among our study patients is therefore not unexpected. In study I, 86% of the CH patients in active period and 78% of the patients in remission were habitual smokers. However, the control group of healthy subjects was not completely matched, ending up with 60% smokers. In study I, glycerol concentrations, evaluated in two hour intervals, did not differ between smokers and non-smokers and for that reason, the control group was pooled. However, in study II we wanted to more thoroughly explore and report the chronically influence of habitual smoking on adipose tissue metabolism. For that reason we evaluated nocturnal lipolysis for a subgroup, consisting of healthy smokers and non-smokers, during short-term nicotine withdrawal. We could not find any difference in interstitial glycerol concentrations between smokers and non-smokers, neither any difference in interstitial glucose levels.

To our knowledge, there are no previous studies on lipolysis in smokers during short-term nicotine withdrawal. The acute effect of tobacco smoking in habitual smokers is a rapid increase in plasma levels of catecholamines, FFA and glycerol and smoking is consequently considered to indirectly stimulate lipolysis via adrenergic mechanisms (136, 137). More recently, studies of adipose tissue and lipolysis in situ have shown that systemic infusion of nicotine to non-smokers stimulates lipolysis, not only via catecholaminergic mechanisms but also directly via local cholinergic receptors (94, 138). However, lipolysis in situ has been found to be unaffected in habitual smokers after smoking one cigarette (139) but it was discussed whether the nicotine dose was too low, since plasma catecholamine levels were unaffected as well. Since our patients were examined during nicotine withdrawal, the acute lipolytic effect of nicotine can be discarded. However, the effects of withdrawal have to be considered. Elevated plasma noradrenaline levels after 48 hours of nicotine abstinence have been reported (140). Accordingly, an increased lipolysis rate would have been expected in the smokers in our study. However, compared with the non-smokers we could not demonstrate any significant alteration of the nocturnal lipolysis after a seven hour pre-trial withdrawal. We investigated lipolysis after rather short tobacco withdrawal and also during night time, a period when the subjects normally would not smoke. This study setting
probably reduces the risk for stress symptoms with elevated noradrenaline levels and increased lipolysis related to the nicotine abstinence *per se*.

A peripheral disturbance in the adipose tissue, such as diminished receptor sensitivity, may also explain why there was no increase of lipolysis in habitual smokers. In support, it is previously shown that smokers, compared to non-smokers, have a reduced density of β-adrenoceptors in lymphocytes as well as diminished secretion of plasma FFA after exercise (141). That would indicate a down-regulation of β-adrenoceptors in the adipose tissue of habitual smokers.

In summary, we could not find any significant differences in basal lipolysis rate between smokers during short-term tobacco withdrawal and non-smokers. We concluded that habitual smoking appears not to be associated with any major long-term alterations in subcutaneous lipolysis, in otherwise healthy subjects and that smoking habits did not explain the diminished nocturnal lipolysis in CH patients.

5.2.3 Study III
Catecholamines stimulate lipolysis by activation of β-receptors in the adipose tissue. Reduced lipolysis, as we found in CH patients, may therefore be a result of diminished β-receptor function. In study III, we measured the lipolytic response to noradrenaline in CH patients in remission, as an estimate of the local β-receptor function in adipose tissue. We found that lipolysis increased rapidly in response to noradrenaline infusion in both CH patients and in healthy subjects, but the average lipolytic response was significantly higher in the CH group.

The present findings do not support a diminished β-receptor function as a cause of reduced lipolysis in CH. Both CH patients and healthy subjects showed a marked lipolytic response to noradrenaline, which successively declined, even though the noradrenaline infusion continued. This finding is in accordance with previous studies and has been interpreted as an acute desensitization of the β-receptors (142, 143). In contrast, a reduced stimulation of the receptors, may lead to a sensitization. Thus, the increased lipolytic response to noradrenaline in CH remission rather indicate an up-regulation of the β-receptors, perhaps as an effect of reduced sympathetic tone.
In experimental studies of healthy subjects, sustained head-down bed rest, which results in sympathetic inhibition, shows increased lipolytic sensitivity to β-receptor agonists (144), as well as an increase of heart rate and vasodilatory responses (145). Indications of up-regulated adrenoceptors have also been observed in disorders with severe autonomic disturbances. In patients with multiple system atrophy (MSA), in whom the autonomic disturbance is considered to be of a central as well as a pre-ganglionic origin, orthostatic hypotension is a major problem. In addition, about 50% of the patients develop hypertension in supine position, probably as a result of residual sympathetic tone acting on hypersensitive, post-synaptic adrenoceptors (146, 147). Chronic autonomic failure could also be a result of ganglionic or postganglionic lesions, as in diabetic peripheral neuropathy. In diabetic patients, with severe autonomic neuropathy, in vitro studies of adipocytes have shown increased β-receptor sensitivity, which in part has been attributed to an increased density of β-adrenergic receptors (148). In analogy, up-regulation of adipose tissue β-receptors in CH, would support a systemic sympathetic hypofunction in this group.

Activation of adrenoceptors influence lipolysis via a stimulatory (Gs) or an inhibitory (Gi) G-protein mediated pathway, acting on the adenylate-cyclase system (85). There are some previous indications of altered adrenoceptor function in CH showing hypofunction of inhibitory G\(_i\)-proteins (149) as well as diminished expression of G\(_i\)\(\alpha\) mRNA in lymphocytes (150). This may result in an altered balance between activating and inhibiting signals to the adenylate-cyclase system. If this is applicable to the \(\alpha\)-receptors in adipose tissue, is not known. A reduced G\(_i\)\(\alpha\) protein activity should result in an enhanced basal lipolysis in the morning. We could not demonstrate that in our CH patients.

In conclusion, an enhanced β-receptor response to noradrenaline, as was seen in CH patients, could be due to sensitization of the β-receptors or alterations of the post-receptor cascade. These findings may support a systemic, sympathetic dysfunction in CH.

5.2.4 Study IV
Lipolysis is stimulated by noradrenaline, GH and to some extent by cortisol, and inhibited by insulin, hormones which are directly and indirectly regulated by hypothalamus. In study IV we found time-related changes in GH concentrations during
CH remission, as shown by a reduced increase in GH between 24.00 and 01.00 hours. Nocturnal secretion of noradrenaline, cortisol and insulin did not differ significantly between CH patients and controls.

_Growth Hormone_

Basal nocturnal GH secretion has never been studied in CH remission before, but in a study during active period a bimodal profile attributed to an advance of the GH evening peak, was reported (32). Dynamic tests with dopaminergic and serotonergic agonists have revealed normal responses (102-105).

If the present findings reflect reduced amplitudes of GH pulses or an altered GH rhythmicity is not possible to determine. Sleep is one of the most important triggers of GH secretion (97) and a sleep disturbance in CH would consequently affect GH release.

There is a relationship between the sleep-wake cycle and CH. CH attacks often occur at night and there is a correlation to REM sleep (151). Moreover, there are some reports of a disturbed sleep pattern in active period of CH (152, 153) and it is also shown that obstructive sleep apnoea (OSA) is more common in CH patients than in the general population (154, 155). Sleep follows a clear circadian rhythm related to the nocturnal melatonin secretion. Melatonin is believed to induce sleep by inhibiting the drive for wakefulness and by induction of phase shifts in the SCN. Reduced excretion of melatonin in urine has been reported in both phases of CH (119), which indirectly may be an indication of a sleep disturbance in CH remission as well. However, in the absence of simultaneous sleep recordings, as in the present study, we do not know whether altered sleep patterns influenced the GH secretory rhythm.

Hypothalamus also influences GH release via GHRH stimulation and somatostatin inhibition. Somatostatin has also been shown to have central pain-modulatory effects and octreotide, a somatostatin analogue, has been shown to be effective in the acute treatment of CH attacks (107). This indicates an involvement of the somatostatin-GH axis in CH pathophysiology, but the role for somatostatin has to be further elucidated. Thus, we cannot exclude an alteration in somatostatinergic tone, as an explanation of our GH findings.
Obesity and age are factors that are well known to reduce GH levels (98). Healthy subjects had slightly higher BMI than CH patients, while CH patients were significantly older than controls. GH production decline exponentially with age. Most of our subjects were between thirty and fifty years old, an interval in which age is not considered to have a major influence on GH secretion. The estimations of GH levels were adjusted for BMI and age respectively, in separate ANOVA models. The statistical estimates of GH were not affected by age, thus the variation in GH between groups was not explained by age differences. BMI was found to correlate with GH pattern and for that reason BMI was included as a covariate in the final model.

**Noradrenaline**

We did not find any differences in noradrenaline nocturnal concentrations or temporal patterns between CH patients in remission and controls. This is in accordance with findings of normal supine daytime noradrenaline levels in CH remission, as well as normal responses to standing up (67), but in contrast to findings in active period that demonstrate decreased plasma levels of noradrenaline in the early morning as well as in the late evening (69). Moreover, both CH patients in active period and in remission have, as an indication of long-term sympathetic function, shown reduced levels of noradrenaline and adrenaline in platelets (70). We hypothesized that diminished lipolysis, as we found in CH patients, is a result of a sympathetic hypofunction and it could be argued that circulating noradrenaline levels should be reduced. Since noradrenaline concentrations in peripheral blood reflect a number of processes involving noradrenaline turn-over, regional changes may not always be detected. Moreover, in central autonomic disturbances, such as multiple system atrophy, circulating catecholamines use to be in normal ranges (156, 157). In analogy, our results with respect to noradrenaline in blood do not exclude a permanent sympathetic hypofunction in CH.

**Insulin**

Serum insulin concentrations did not differ between CH patients and healthy subjects and there are no previous studies of insulin levels or glucose regulation in CH indicating a disturbed insulin regulation. Still, it is possible that insulin related mechanisms may be involved in CH pathophysiology since it has been reported that eight of nine patients in active cluster period developed a typical CH attack, during
insulin tolerance test (37). However, vasodilation due to hypoglycemia was discussed as an attack provoking factor.

**Cortisol**

Cortisol levels or secretory pattern did not differ between CH patients and controls, indicating a normal HPA-axis in CH remission. There is only one previous study, in which nocturnal cortisol has been measured in remission, and our results confirm these findings (34). However, basal morning levels have been found to be increased in remission and CRH- test as well as insulin induced hypoglycemia have revealed blunted cortisol responses in CH remission (37).

**Hormones and lipolysis**

We hypothesized that diminished lipolysis, as we found in CH patients, is a result of a sympathetic hypofunction. In the present study, we did not find any differences in noradrenaline concentrations between CH patients in remission and healthy subjects, which in analogy with previous discussion do not exclude a permanent sympathetic hypofunction in CH.

However, other hormones have effect on lipolysis as well. Nocturnal secretion of GH was demonstrated to be altered in CH patients in remission. GH is known to stimulate lipolysis in a slower way than catecholamines (86, 88, 158) but the effect is mostly permissive by improving the sensitivity for catecholamine action (89, 90). A blunted GH peak around midnight may consequently influence lipolysis later in the night. For that reason, the alterations in lipolysis levels and rhythm that was found in patients in remission might be a result of diminished GH release or a dyssynchronization between GH and catecholamine secretion. If an altered GH pattern is influencing lipolysis in active period as well, is still unclear.

There were no alterations in secretion of insulin and cortisol, which indicates that they are not involved in the altered lipolysis in CH.

In conclusion, there are indications of an altered nocturnal GH secretion in CH remission, which further would support a hypothalamic disturbance in CH. An altered GH secretion may in part explain the reduced lipolysis previously found in CH remission.
6 GENERAL CONCLUSIONS

Diminished nocturnal lipolysis was shown in CH patients, both in active period and in remission. In addition, patients in remission showed altered nocturnal lipolysis rhythmicity. These findings demonstrate for the first time a permanent metabolic disturbance in CH, which we hypothesized to be a result of a systemic, sympathetic hypofunction, possibly at a hypothalamic level.

We could demonstrate that altered lipolysis in CH was not explained by long-term smoking habits, nor by defective β-receptors in the adipose tissue. On the contrary, β-receptors in adipose tissue appeared to be up-regulated, in support of our hypothesis of a decreased sympathetic tone and systemic sympathetic dysregulation in CH.

We also investigated if a diminished lipolysis in CH could be a result of altered secretion of noradrenaline, GH, insulin or cortisol, hormones with a potential effect on lipolysis and which at least in part are regulated by hypothalamic activity. We found altered GH concentrations during the early part of the night in CH but a normal secretion of noradrenaline, insulin and cortisol. We suggested that the altered nocturnal GH pattern in CH remission in part may explain the altered nocturnal lipolysis in CH, and also supports a permanent hypothalamic disturbance in CH.
7 FUTURE PERSPECTIVES

In this thesis, we showed a relationship between adipose tissue metabolism and CH. It is a clinical impression that CH patients may have a less body-mass index than age matched controls and for that reason it would be of interest to further study hypothalamic systems involved in feeding behavior and energy balance.

Hormones with potential interest may be leptin, which is released from adipose tissue, as well as several gastro-intestinal hormones that via parasympathetic pathways project on receptors in the hypothalamus and influence hunger and satiety.
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8 REFERENCES


