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THE VASOPRESSIN SYSTEM IN DIABETES MELLITUS, OBESITY AND THE METABOLIC SYNDROME

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2012

Citation for published version (APA):
Enhörning, S. (2012). *THE VASOPRESSIN SYSTEM IN DIABETES MELLITUS, OBESITY AND THE METABOLIC SYNDROME*. [Doctoral Thesis (compilation), Cardiovascular Research - Hypertension]. Hypertension and Cardiovascular Disease.

Total number of authors:
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THE VASOPRESSIN SYSTEM IN DIABETES MELLITUS, OBESITY AND THE METABOLIC SYNDROME

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"If we knew what it was we were doing, it would not be called research, would it?"

Albert Einstein, 1879-1955

"Ta ut glädjen i förskott, annars kanske det inte blir någon"

Emil Jensen, 1974-

"I'm glad I did it, partly because it was worth it, but mostly because I shall never have to do it again"

Mark Twain, 1835-1910

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II *Plasma copeptin and the risk of diabetes mellitus.* Enhörning S, Wang TJ, Nilsson PM, Almgren P, Hedblad B, Berglund G, Struck J, Morgenthaler NG, Bergmann A, Lindholm E, Groop L, Lyssenko V, Orho-Melander M, Newton-Cheh C, Melander O. Circulation. 2010 May 18;121:2102-8.

III *Plasma copeptin, a unifying factor behind the metabolic syndrome.* Enhörning S, Struck J, Wirfält E, Hedblad B, Morgenthaler NG, Melander O. J Clin Endocrinol Metab. 2011 Apr 13. 96:E1065-72.

IV *Copeptin, a marker of vasopressin, predicts abdominal obesity, diabetes and microalbuminuria: the prospective Malmö Diet and Cancer study cardiovascular cohort.* Enhörning S, Bankir L, Bouby N, Struck J, Hedblad B, Persson M, Morgenthaler NG, Nilsson PM, Melander O. Int J Obes. E-pub ahead of print 22 May 2012; doi: 10.1038/ijo.2012.88.

V   *Genetic Vasopressin 1b Receptor variance in overweight and diabetes mellitus.* Enhörning S, Sjögren M, Hedblad B, Nilsson PM, Struck J, Melander O. Submitted.

ABBREVIATIONS

ACTH   adrenocorticotrophic hormone
AHT   anti-hypertensive treatment
AVP   arginine vasopressin
AVPR1A   arginine vasopressin receptor 1a gene
AVPR1B   arginine vasopressin receptor 1b gene
BMI   body mass index
BP   blood pressure
CI   confidence interval
CRH   corticotropin-releasing hormone
CRP   C-reactive protein
CVD   cardiovascular disease
DM   diabetes mellitus
FBG   fasting blood glucose
FPG   fasting plasma glucose
GFR   glomerular filtration rate
HbA1c   hemoglobin A1c
HDL   high density lipoprotein cholesterol
HOMA-IR   homeostatic assessment model of insulin resistance
HPA   hypothalamic-pituitary-adrenal
IDI   integrated discrimination improvement
IFG   impaired fasting glucose
LDL   low density lipoprotein cholesterol
MDC   Malmö Diet and Cancer study
MDC-CC   Malmö Diet and Cancer study cardiovascular cohort
MetS   the metabolic syndrome
MHR   Malmö HbA1c register
NDR   national diabetes register
OGTT   oral glucose tolerance test
ROC curve   Receiver Operating Characteristic curve
SD   standard deviation
SNP   single nucleotide polymorphism
SPSS   statistical package for the social sciences

TG	triglycerides
V1aR	vasopressin 1a receptor
V1bR	vasopressin 1b receptor
V2R	vasopressin 2 receptor
WHO	World Health Organization

INTRODUCTION

The main purpose of epidemiology is to improve health in a specified population, which is achieved by its ability to identify and map determinants and distribution of disease. The Greek physician Hippocrates is considered to be the first epidemiologist as he observed the influence of environmental factors on infectious disease about 2500 years ago [1]. However, it was not until the 19th century epidemiological methods began to be used more extensively, mostly to determine the distribution of infectious diseases. During the second part of the 20th century, the focus of epidemiology has shifted from infectious diseases to chronic diseases.

Chronic diseases are currently the major cause of death in almost all countries, accounting for approximately 35 million deaths around the world each year (60% of the total mortality). The predominant chronic disease groups are cardiovascular diseases (which accounts for approximately 50% of all chronic disease deaths), cancer, chronic respiratory diseases and diabetes mellitus [2]. Due to aging population, urbanization and changing lifestyle, the incidence of obesity and diabetes is growing worldwide [3]. The prevalence of obesity and overweight among the world's adult population is predicted to rise from 33% (1.3 billion people) in 2005 to 57.8% (3.3 billion people) in 2030 [4], and the world prevalence of diabetes among adults is estimated to rise from 6.4% in 2010 (285 million people) to 7.7%, (439 million people) by 2030 [5]. These figures indicate a rapidly developing cardiovascular disease epidemic.

This thesis consists of a number of epidemiological studies investigating the role of the vasopressin hormonal system in body weight control and glucose metabolism. Vasopressin is previously sparsely mentioned in this context, but is more commonly known as an important operator in the salt-water regulation of the body.

The main purpose of epidemiology is to improve health, and the main purpose of this thesis has been to further explore the role of the vasopressin system in the pathophysiology behind metabolic disorders, in this way enabling health improvement in the fields of diabetes and overweight by identifying the vasopressin system as a new possible treatment target.

DIABETES

Definition and diagnosis

Diabetes mellitus (DM) is a heterogenous metabolic disease characterized by hyperglycemia induced by defect insulin secretion, insulin action, or both. DM is diagnosed either when symptoms of hyperglycemia (polyuria, polydipsia and unexplained weight loss) are accompanied by a casual plasma glucose ≥11.1 mmol/L, or if the fasting plasma glucose is equal to or exceeds 7.0 mmol/L, or if the 2-h glucose is ≥11.1 mmol/L during an oral glucose tolerance test (using a load of 75 g glucose), or if HbA1c is ≥6.5% according to the US National Glycohemoglobin Standardization Program (NGSP) [6]. In absence of obvious hyperglycemia, these three latter criteria should be confirmed by repeated testing another day. The HbA1c criteria was just recently established [6]. Historically, blood glucose was often measured in whole blood. DM is diagnosed in a fasting whole blood sample when glucose value is equal or exceeds 6.1 mmol/L, corresponding to fasting plasma glucose of 7.0 mmol/L. The lower cut-off level of glucose for DM diagnosis in whole blood is explained by the relatively low content of free water inside red blood cells (70%), in comparison to plasma that has got a relatively higher content of free water (90%), and consequently more dissolved glucose, when compared to whole blood.

Impaired fasting glucose (IFG) is a term describing the intermediate stage between normal glucose metabolism and DM. Subjects with IFG have an increased risk of developing DM and cardiovascular disease compared to normoglycemic subjects, emphasizing the importance of early interventions [7]. IFG is currently defined (by the American Diabetes Association) as fasting plasma glucose between 5.6 and 6.9 mmol/l [6, 7]. However, it should be added that the World Health Organization (WHO) among other organizations have not accepted this directive and still uses the cut point of fasting plasma glucose ≥6.1 mmol/l, corresponding to fasting whole blood glucose ≥5.4 mmol/l, to define FPG [8].

DM is usually subdivided according to different etiological backgrounds of the disease. Type 1 DM is an autoimmune disease which often occurs in early years. It causes β-cell destruction in the islets of Langerhans leading to insulin deficiency [9].

Type 2 DM is a condition of insulin resistance and relative insulin deficiency [9]. It is a complex disorder where the underlying causes are a mixture of environmental factors such as age, low physical activity and obesity [10], and genetic predisposition, which determines the individual susceptibility to type 2 DM. Genetic variation in several genes has been

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associated with increased DM risk [11]. The disease often remains undiagnosed for a long time as the hyperglycemia with its symptoms develops gradually.

In addition to type 1 and type 2 DM, there are other specific types of DM. Maturity onset diabetes of the young (MODY) is inherited in an autosomal dominant mode and is caused by specific mutations affecting β-cell function leading to impaired insulin secretion and hyperglycemia in early age. MODY accounts for about 2-5% of all DM cases [12]. Gestational DM is defined as glucose intolerance with onset or first recognition during pregnancy. Six weeks or more after the end of pregnancy, the glucose tolerance should be re-examined, and in most cases, it will have returned to normal [13]. The diverse group of other specific types of DM also includes DM associated with complex genetic syndromes, for example Down's syndrome and Turner's syndrome.

Epidemiology

The cause of the epidemic increase in prevalence of type 2 DM is probably not found in our genes, but is most likely a result from rapid changes in the environment, including caloric over-consumption and decreasing physical activity, which triggers disease development in genetically predisposed individuals [14]. The prevalence of DM worldwide in year 2010 among adults (20-79 years of age) was estimated to be 6.4%, affecting 285 million adults, and is expected to rise to 7.7%, and 439 million adults by 2030 [5]. DM is associated with premature death. It was estimated that almost 4 million deaths (6.8% of global all-cause mortality) were due to DM in 2010 [15]. Furthermore, the dramatic increase in prevalence of DM leads to multiple complications and high healthcare costs. The late complications of DM cause mortality and morbidity including damage and failure of several organs. The microvascular damage in DM leads to retinopathy, neuropathy and nephropathy. However, most of the morbidity and mortality associated with diabetes is caused by macrovascular complications, for example myocardial infarction and stroke [16]. The causes of the interaction between DM and cardiovascular diseases (CVD) are incompletely understood, and studies has not been able to show that improvement of glucose control in DM leads to decreased risk of CVD mortality [17]. Thus, it is relevant to find pharmacologically modifiable factors other than glucose level that are linked to CVD in DM patients.

OVERWEIGHT AND OBESITY

Definition and diagnosis

Overweight is the product of a positive energy balance resulting from high energy intake and low physical activity, factors which to a high extent are determined by our social and cultural environment. However, the predisposition to excess weight gain is also determined by our genes. As limited access to food and high physical activity was the dominating condition during human evolution, genes contributing to fat storage and weight gain previously were beneficial for survival during times of energy deficit. However, the genetic basis of overweight and obesity is complex, and many genes are involved [18, 19]. Genes may affect both food intake and energy expenditure, but in most cases it is unclear through which mechanisms genes influence weight gain. The genetic contribution to obesity is estimated to control between 25 and 40% of the individual differences in BMI [20].

Body mass index (BMI) is an index which relates weight to height, commonly used to classify overweight and obesity. It is defined as the weight in kilograms divided by the square of height in meters (kg/m^2). Overweight is defined as body mass index (BMI) ≥ 25 kg/m^2, and obesity as BMI ≥ 30 kg/m^2 [21].

During recent years more attention has focused on the pattern of body fat distribution. It is suggested that measures of abdominal obesity (central adiposity) are a more important determinant of CVD risk than BMI [22, 23]. Initially waist to hip ratio, and later on waist circumference were used to measure abdominal obesity. Waist circumference is related to the risk of metabolic and cardiovascular diseases and correlates well with the amount of visceral fat [24, 25], and this measure has become widely used as a proxy for abdominal fat deposition. Abdominal obesity is defined as waist circumference >102 cm in men and >88 cm in women [26, 27].

Epidemiology

The worldwide increase in overweight and obesity that has taken place during the past 30 years can be explained by less physical activity in parallel to change in dietary patterns. The prevalence of overweight or obesity among the world's adult population is predicted to rise from 33% in 2005 to 57.8% in 2030, if recent secular trends of obesity continue[4]. Obesity is associated with increased mortality, mainly due to CVD [28] and cancer [29], whereas weight loss leads to decreased overall mortality [30]. Furthermore, elevated BMI is the single most important predictor of type 2

DM. The relative risk of DM was 38.8 for women with a BMI of 35.0 or higher, and 20.1 for women with a BMI of 30.0 to 34.9, as compared with women who had a BMI of less than 23.0 [31]. Thus, it is crucial for public health to prevent further increase of the world's prevalence of obesity.

THE METABOLIC SYNDROME

Definition and diagnosis

The metabolic syndrome (MetS) is a cluster, within the same individual, of cardiometabolic risk factors, including hypertension, abdominal obesity, dyslipidemia, insulin resistance and proinflammatory and prothrombotic states. There are several different definitions of MetS, but all of them include insulin resistance or glucose intolerance, hypertension, dyslipidemia and central obesity. The most commonly used definitions are summarized in Table 1.

The diverse clinical characteristics, involving several metabolic pathways, illustrate the complexity of the MetS. Its multifactorial etiological background is poorly understood, and the link between the different MetS components is still unknown. However, the components co-occur in an individual more often than would be expected by chance [32], and in addition to genetic and environmental factors contributing to each individual MetS component, it is suggested that there is a single unifying etiological factor contributing to the entire cluster [33]. Knowledge of the underlying causes of MetS is important, and identification of basic pathogenic mechanisms unifying the MetS components would be helpful for identification of possible life-style and pharmacological treatment.

Increased urinary albumin excretion is a risk factor for CVD. Microalbuminuria was in the WHO definition from 1998 initially considered as a component of the MetS [9], but was later on removed as a criterion (Table 1) as it was undecided whether microalbuminuria by itself contributed with any value on top of the other MetS variables in the prediction of CVD, or if microalbuminuria was just a secondary response to the vascular damaging disease processes [34]. However, studies show that albuminuria indeed contribute with predictive information in addition to the MetS in prediction of CVD [35, 36].

Inflammatory variables, for example c-reactive protein (CRP), have been proposed as a component of the metabolic syndrome [37]. Likewise in the discussion concerning microalbuminuria, controversy exists as to whether CRP contributes to MetS, or if it just reflects the inflammatory disease processes of MetS. Anyhow, several studies have shown that CRP adds prognostic value on top of the MetS in CVD prediction [35, 38]. This suggests that albuminuria and CRP may be valuable additions to the current MetS definition.

Epidemiology

The relatively high prevalence of MetS, with about one out of four in the adult population affected [39, 40], is a worldwide phenomenon, and the prevalence is suggested to rise because of a parallel worldwide increase in the prevalence of obesity [40]. MetS is associated with increased risk of CVD and mortality [36, 39, 41]. It is estimated that individuals with MetS are at essentially twice the risk for CVD compared with those without MetS [40].

As mentioned above, the underlying pathophysiology of MetS is unclear. Furthermore, it remains unclear whether or not MetS is useful for estimating risk for CVD beyond the risk associated with its individual components [41]. Nevertheless, MetS is a tool currently used by clinicians as well as researchers, and is therefore of interest.

Table 1. Summary of different definitions of the metabolic syndrome.

	WHO (1998)	NCEP ATP III (2001)[1]	IDF (2005)
Prerequisite	Impaired glucose metabolism, T2DM or insulin resistance.	None	Waist circumference
General criteria	As above and at least two other.	Three or more of the following:	As above and at least two other.
Waist circumferenc/ obesity	BMI≥30 and/or WHR>0.9 in men, >0.85 in women	>102cm in men, >88cm in women	≥94cm in men[2], ≥80cm in women[2]
Triglycerides (mmol/l)	≥1.7	≥1.7	≥1.7
HDL (mmol/l)	<0.9 in men, <1.0 in women	<1.0 in men, <1.3 in women	<1.0 in men, <1.3 in women
Blood pressure	≥140 systolic and/or ≥90 diastolic or AHT	≥130 systolic and/or ≥85 diastolic or AHT	≥130 systolic and/or ≥85 diastolic or AHT
Glucose/ insulin resistance	FPG ≥6.1 mmol/l or IGT or type II DM or lowered insulin sensitivity[3]	FPG ≥6.1 mmol/l	FPG ≥5.6 mmol/l
Microalbumin- uria	Urinary AER ≥20μg/min		

Abbreviations: AER, albumin excretion rate; AHT, anti-hypertensive treatment; BMI, body mass index; FPG, fasting plasma glucose; HDL, high density lipoprotein cholesterol; HOMA, homeostasis assessment model; IDF, International Diabetes Federation; IGT, impaired glucose tolerance; MetS, metabolic syndrome; NCEP ATP III, National Cholesterol Education Program Adult Treatment Panel III; T2DM, type 2 diabetes mellitus; WHR, waist to hip ratio.

[1] The 2001 ATP III definition identified impaired fasting plasma glucose (IFG) of 6.1 mmol/l as elevated. In line with the American Diabetes Association's updated definition [7], the ATP III definition of MetS was modified in 2004, identifying IFG of 5.6 mmol/l as elevated. We use the old ATP III definition of MetS in this thesis, in consistency with the use of WHO's definition of IFG (fasting plasma glucose ≥ 6.1 mmol/l, corresponding to fasting whole blood glucose ≥5.4 mmol/L).

[2] Population-specific cut-off levels, these values apply only to Caucasians.

[3] Insulin sensitivity, measured under hyperinsulinemic euglycemic conditions, defined as glucose uptake below lowest quartile for background population under investigation. IGT defined as 2-h glucose value between7.8 and 11.0 mmol/l during oral glucose tolerance test.

Adapted from [42] and [43].

THE VASOPRESSIN SYSTEM

Arginine vasopressin (AVP), also known as antidiuretic hormone, is a vasopressor and antidiuretic peptide released from the posterior pituitary gland (neurohypophysis) in conditions of increased plasma osmolality or decreased blood pressure. The peptide is called arginine vasopressin since it was previously necessary to distinguish from the lysine vasopressin that was extracted from pigs and used before the synthetic AVP hormone became accessible [44].

AVP is short-lived in plasma, with a mean half-life of 24 minutes [45]. It exerts an antidiuretic effect in the kidney and vasoconstrictive and blood platelet aggregating effects in the vessels. A synthetic analogue of AVP, desmopressin, has been used clinically over the past decades to treat diabetes insipidus and von Willebrands disease [46, 47]. Furthermore, AVP induces gluconeogenesis and glycogenolysis in the liver [48, 49], insulin- and glucagon release by the Langerhans islets of the pancreas [50], and ACTH release from the anterior pituitary gland [51].
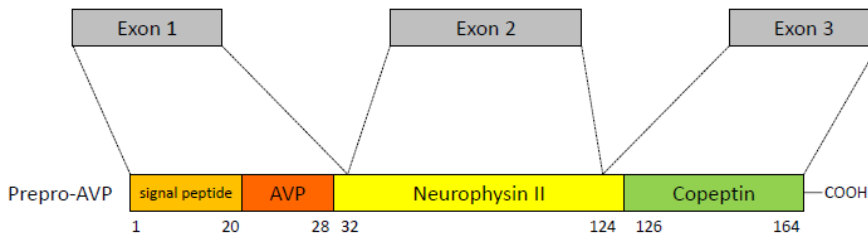
Figure 1. Numbers indicate amino acids. AVP is synthesized as part of a larger precursor protein, prepro-AVP. The AVP gene, encoding prepro-AVP, is located at chromosome 20. Exon 1 of the gene encodes the signal peptide of 19 amino acids, the nonapeptide AVP and the N-terminal region of neurophysin II. Exon 2 encodes the central region of neurophysin II. Exon 3 encodes the C-terminal region of neurophysin II and a 39 amino acid glycopeptide known as copeptin. Figure adapted from [46].

The AVP precursor protein, prepro-AVP (Figure 1), is synthesized in magnocellular hypothalamic neurons and packaged into neurosecretory granules. During axonal transport to the posterior pituitary, Prepro-AVP is cleaved into the product peptides: AVP, neurophysin II and copeptin [46]. Neurophysin II is a carrier protein that serves to stabilize AVP during transport and storage, and help in the correct folding and targeting of the AVP precursor [46, 52]. Copeptin is also suggested to play a role in the correct folding and maturation of the AVP precursor [53]. All three

peptides are released from the posterior pituitary after hemodynamic or osmotic stimuli [54]. In its structure, AVP very much resemble Oxytocin, the other posterior pituitary gland hormone. Oxytocin is synthesized via a similar precursor as AVP, but which lacks copeptin [53].

AVP is released from the posterior pituitary when neurons in hypothalamus are depolarized by osmoreceptor or baroreceptor stimuli [46]. The osmoreceptors, neurons in lamina terminalis, are excluded from the blood brain barrier and thus affected by changes in the concentration of systemic fluid solutes [51], thereby stimulating both thirst and AVP secretion in condition of increased plasma osmolality. Sodium and its anions normally represent more than 95% of the osmotically active solutes in plasma [55] and is the most powerful solute to stimulate AVP release.

AVP release is said to be suppressed to undetectable levels below a certain threshold level of osmolality, usually below 280 mosmol/l [56, 57], but this so called suppression may be caused by inability to measure AVP when the concentration is below a certain level [44]. Above the threshold level, AVP is released proportionally to an increase in plasma osmolality (Figure 2).
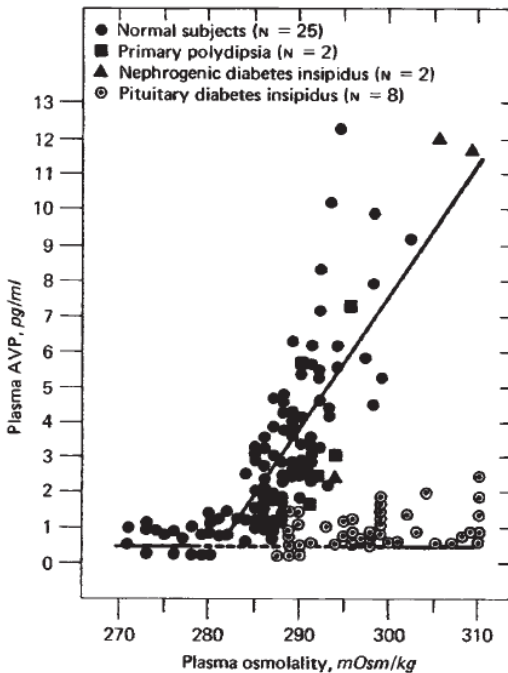
Figure 2: AVP increases proportionally to the increase in plasma osmolality. Subjects with pituitary diabetes insipidus have an impaired AVP secretion, and subjects with nephrogen diabetes insipidus have an impaired AVP function in the renal collecting duct. Reprinted by permission from Macmillan Publishers Ltd: Kidney International, Robertson, G.L., The osmoregulation of vasopressin. 1976. 10: p. 25-37. Copyright ©1976.

Surprisingly, osmoreceptors are not capable of responding to all solutes. For example a rapid infusion of hypertonic glucose does not contribute to the osmotically driven AVP release, but on the contrary it leads to decreased AVP levels in plasma (Figure 3). This selectivity in osmoreceptor response is speculated to be due to the rate at which different solutes penetrate the blood brain barrier. Glucose passes the barrier very quickly whereas sodium penetration is much slower [55].
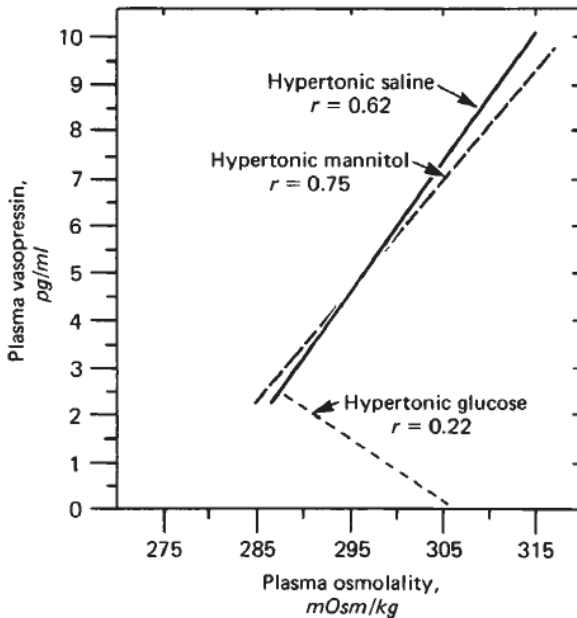
Figure 3: In healthy adults, sodium and mannitol, which both penetrate the blood brain barrier very slowly, contribute to the osmotically driven AVP release, whereas rapid infusion of hypertonic glucose, which penetrates the blood brain barrier quickly, leads to decreased AVP levels in plasma. In each case the rates of change in osmolality and expansion of blood volume were the same. Reprinted by permission from Macmillan Publishers Ltd: Kidney International, Robertson, G.L., The osmoregulation of vasopressin. 1976. 10: p. 25-37. Copyright © 1976.

In addition to the osmotically driven AVP release, increased baroreceptor signaling mediates AVP secretion by depolarization of hypothalamic neurons. The baroreceptors are located at the aortic arch and the carotid sinuses and provide excitatory input to hypothalamic neurons in conditions of low blood pressure [58]. The afferent nerve impulses travel from the baroreceptors via the vagus nerve to nuclei in hypothalamus [51]. The baroreceptor reflex serves to stabilize perfusion pressure [58], but the influence of AVP on blood pressure control is complex and not well defined in humans. It is thought that AVP during normal conditions have a minor role in blood pressure regulation, but that the influence of AVP

increases in response to hypotension, for example in states of hemorrhage or sepsis [59]. Baroreceptor-mediated AVP secretion is speculated to maintain perfusion pressure by modulating vasoconstriction, and the increased vasoconstriction is probably the result of both direct effects of AVP on vascular smooth muscle cells as well as activating effects of AVP on the renin-angiotensin system [60-63].
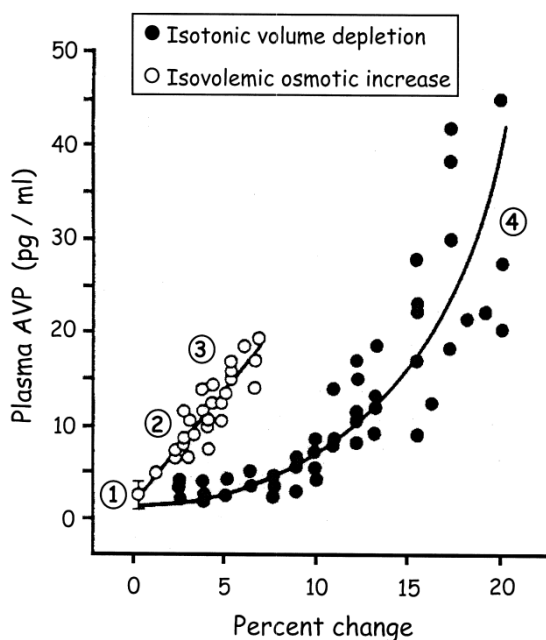
Figure 4: Percental increase in plasma osmolality and percental decrease in blood volume as stimuli for progressive AVP secretion in rat. Levels 1 and 2 correspond to moderate osmotic stimulation occurring in usual life. Level 3 corresponds to mild dehydration. Higher values of plasma AVP (level 4) usually occurs after severe dehydration or reduced extracellular fluid volume. Changes in blood volume were achieved by injection of intraperitoneal polyethylene glycol, leading to reductions in blood volume without altering plasma osmolality. Reprinted by permission from Oxford University Press: Cardiovascular research, Bankir, L., Antidiuretic action of vasopressin: quantitative aspects and interaction between V1a and V2 receptor-mediated effects. 2001. 51: p. 372-90 Copyright © 2001.

Under normal conditions, small changes in osmolality are more potent than small changes in blood volume in affecting AVP levels; the blood volume needs to be reduced by about 9% before a significant rise in AVP is seen (Figure 4). However, very high levels of AVP are more easily produced by reducing blood volume than by raising osmolality (Figure 4) [64]. Thus, higy levels of AVP are observed in conditions of low perfusion pressure and in states of reduced effective arterial blood volume, for example heart failure or cirrhosis [64-66]. In these states, the nonosmotic (baroreceptor) factors overrule the osmotic factors, leading to AVP release in conditions of hyponatremia and hypo-osmolality.

The vasopressin receptors and functions

AVP acts in many organs and has a variety of different physiological effects mediated through three AVP receptors – the V1a receptor (V1aR), V1b recptor (V1bR) and V2 receptor (V2R). The receptors are G protein coupled. The V2R uses cyclic AMP as second messenger, whereas V1aR and V1bR use calcium as second messenger [67].

The V1aR is widely expressed in the body. In humans, the receptor is for example found in the brain, the smooth muscle cells, the liver and the kidney [68]. In mice, V1aR is also expressed in white and brown adipose tissue [69]. V1aR mediates vasoconstriction and platelet aggregation in the blood vessels [63, 70] and glycogenolysis and gluconeogenesis in the liver [48, 49].

Both V1aR and V1bR are expressed in the brain. The V1aR is suggested to have an important role in the physiology of behavior. Variation in the human V1aR gene (AVPR1A) has been associated with diverse behavior in social interaction and partner bonding, and may be a contributor to behavioral deficits associated with autism [71].

The V1bR is a component of the hypothalamic-pituitary-adrenocortical (HPA) axis. Except for the axonal transport of Prepro-AVP to the posterior pituitary, AVP together with copeptin, as well as corticotrophin-releasing hormone (CRH), is produced and released from parvocellular neurons in the hypothalamus directly into the pituitary portal capillaries of the median eminence (which is the part of the hypothalamus from which regulatory hormones are released) and transported to the anterior pituitary [54, 72]. In the anterior pituitary V1bR is expressed, and mediates adreno-corticothrophic hormone (ACTH) release upon stimulation of AVP. This AVP mediated control is, together with the CRH mediated effects, important for the maintenance of ACTH and corticosterone levels in the endocrine stress response [72, 73]. In addition to the participation in the HPA axis, V1bR is expressed in other structures in the brain, for example hippocampus, amygdala and prefrontal cortex, where it is considered to be involved in memory, recognition, anxiety and depression [74].

Furthermore, V1bR is expressed in the pancreas where it mediates glucagon or insulin secretion, depending on the current glucose level in plasma [50].

AVP exerts various effects in the kidney through both V1aR and V2R. The major actions of AVP in the kidney occur in the collecting ducts where V2R mediates increased water permeability by recruitment of aquaporin type 2 [75, 76]. Furthermore, V2R activation mediates reabsorption of NaCl in the thick ascending limb [77, 78]. V1aR is mainly localized in the renal vasculature, glomeruli, and the collecting duct [79]. An intricate interaction

between V1aR and V2R mediated effects in the kidney is suggested, in which V1aR is proposed to play an antagonistic role [44].

In addition to the three above mentioned AVP receptors, the peptide hormone oxytocin, which is structurally very similar to AVP, also have a receptor that can bind AVP [80].

The contribution of the AVP system to glucose and fat metabolism

There are several studies in which the data support that the AVP system is involved in the control of fat and glucose homeostasis. AVP mediates gluconeogenesis and glycogenolysis through V1aR in the liver [48, 49] and stimulates the secretion of either glucagon or insulin, depending on the actual level of glycemia, through V1bR in pancreatic islets [50]. Moreover, elevated AVP levels are observed in subjects with uncontrolled diabetes mellitus [81], and AVP infusion in healthy subjects leads to increased blood glucose levels and a concomitant rise in glucagon levels [82].

V1bR mediated ACTH release elevate glucocorticoid levels in plasma [72, 73]. This AVP-induced ACTH release is reported to be resistant to glucocorticoid feedback in contrast to CRH induced ACTH release [83], which potentially could lead to altered glucose and fat metabolism.

Mice with selective deletion of V1aR exhibit elevated glucose levels, predisposition for obesity and DM and elevated AVP levels compared with wild type mice [84]. On the contrary, mice lacking V1bR display a phenotype of low glucose levels and better insulin sensitivity compared with wild type mice [80].

When it comes to AVP induced alterations of fat metabolism, the mechanisms responsible are less studied than the mechanisms controlling glucose homeostasis. In mice, V1aR and V1bR are expressed in adipose tissue [69]. In rats, AVP exerts an anti-lipolytic action, possibly through haemodynamic effects [85], and mice with selective deletion of V1aR express a phenotype of low TG levels and enhanced lipid metabolism [69]. In humans, a polymorphism of AVPR1A has been associated with altered BMI [86]. Finally, among subjects with hypopituitarism, obesity is more prevalent in subjects with AVP deficient hypopituitarism and treatment with the highly V2-selective AVP analogue desmopressin, than in subjects with other types of hypopituitarism [87].

Copeptin

AVP is short-lived in plasma and most AVP assays have relatively limited sensitivity [45]. Furthermore, because of its small size, it is not possible to detect AVP by sandwich immunoassays [88]. Thus, an assay has been developed to quantify AVP release by measurement of plasma copeptin (copeptin), the C-terminal stable cleavage product of the AVP precursor protein (Figure 1). Copeptin is considered to be a clinically useful surrogate marker for AVP as it correlates well with AVP levels and can be determined quantitatively and reliably in plasma [88, 89]. AVP is eliminated mainly by glomerular filtration [45]. It is considered that also copeptin is cleared from plasma by the kidney [90], and copeptin values correlates with estimated glomerular filtration [91].

Copeptin levels in healthy volunteers generally ranges between 1 and 12 pmol/L with median values <5 pmol/L, and median values are about 1 pmol/L higher in men [54, 88, 91]. Its physiologic function remained unknown for a long time, but recently copeptin was suggested to have an important role in the structural formation of the AVP precursor [53]. Copeptin levels does not correlate with age [91].

So far, copeptin has been associated with a range of clinical states. Elevated copeptin levels are observed in response to hemorrhagic and septic shock [92] and pneumonia [93, 94]. In subjects with polycystic kidney disease, and in renal transplant recipients, copeptin is a predictor of renal function decline [95, 96]. Furthermore, copeptin is proposed as a useful prognostic marker in heart failure [97], stroke [98, 99], myocardial infarction [100] and diabetic heart disease [101], and in the acute setting copeptin can rule out myocardial infarction [102-104].

GENETICS

Complex and polygenetic diseases

Diabetes, obesity and MetS are all common complex diseases. The underlying causes are a mixture of environmental factors and genetic predisposition. It is a major challenge to find the genes that affect disease susceptibility for complex diseases. Unlike rare mendelian diseases, in which rare gene mutations have great impact on disease susceptibility, the genes that give rise to common complex diseases are many, but the individual contribution to development of disease of each gene is small. For example, until now more than 40 type 2 DM-associated genes have been identified, but these genetic variations have a modest impact on disease development, and altogether they explain less than 10% of the type 2 DM heritability [11]. Furthermore, the interaction between genetic variants and environmental factors may be important in determining disease susceptibility.

Genetic variance and association studies

Genetic variance is the variation in DNA sequence from one individual to another. Most of this variation constitutes of single base pair substitution (single point mutation) called single nucleotide polymorphism (SNP). To be considered as a SNP, the substitution must occur in at least 1% of the population [105].

Many people share a chromosome segment inherited from a distant common ancestor who carried a susceptibility factor. By using catalogued genetic markers, i.e. SNPs, these ancestral chromosome segments can be defined [106]. This means that the SNPs are used to find out if someone has inherited a certain gene that increases disease susceptibility.

A haplotype is a sequence of consecutive alleles on a chromosome, and the SNPs within this sequence are associated (they are in linkage disequilibrium). In the online catalogue Hap Map (Haplotype map)[107] the ancestral chromosome segments of four human populations have been defined, and catalogued tag SNPs are used to identify them. Tag SNPs are SNPs that correlate with all the other SNPs in the same chromosome segment. They are selected to represent the maximum of the genetic variation. Usually, SNPs separated by a large distance on the chromosome are not well associated with each other. This is due to the recombination that occurs during meiosis, i.e. in each generation, mixing the allele sequences of the two homologous chromosomes by cross-overs [106].

The prime method to find the genetic architecture behind complex diseases is association analysis which tests for differences in allele frequencies between affected subjects and non-affected subjects [108]. The underlying principle for association studies is the "common disease, common variant" hypothesis, that common diseases are caused by common genetic variants, present in more than 1% of the population, and not rare genetic variants present in less than 1% of the population [109].

If SNPs are examined in a certain gene, which was chosen on the basis of known biological function, this approach is called candidate gene approach. The method has been widely used, but the results have often been difficult to replicate [110]. In the search of genetic risk factors behind type 2 DM, hundreds of candidates genes have been investigated, but only a few genes such as PPARγ and TCF7L2 have repeatedly been found to be associated with type 2 DM [11]. Today, replication of findings in independent data sets is regarded as a prerequisite for evidence of association [111].

Recently it has become feasible to examine hundreds of thousands of SNPs throughout the genome for association with disease, in thousands of individuals [109]. These genome-wide association studies (GWAS) examine the link between disease and common variation in the human genome without a prior hypothesis of which genes that may play a role in the pathophysiology behind disease. In this way, GWAS are hypothesis generating. The great majority of known type 2 DM susceptibility genes that are known today have been identified by GWAS technique.

AIMS

The general aim of this thesis was to gain further knowledge of the role of the vasopressin system in the pathogenesis of DM, weight gain and MetS.

The specific aims were:

I. To evaluate the role of human AVPR1A gene variance in glucose and fat metabolism.

II. To explore the associations between copeptin and development of DM in a register-based follow-up.

III. To investigate if copeptin is associated cross-sectionally not only with DM but also with other components of the MetS.

IV. To evaluate the link between baseline copeptin and development of components of the metabolic syndrome at reinvestigation, in this way also validating previous register-based DM findings.

V. To explore the role of human AVPR1B gene variance in glucose and fat metabolism.

METHODS

POPULATION AND COHORT DESCRIPTION

The Malmö Diet and Cancer study (MDC) is a population-based prospective cohort consisting of 30447 individuals surveyed in 1991-1996 with the aim of exploring the links between dietary patterns and cancer (Figure 5) [112]. In the city of Malmö, the third largest city of Sweden (235000 inhabitants in 1991), all men born between 1923 and 1945 and women born between 1923 and 1950 were recruited. In total, there were 74138 individuals in the selected birth cohorts according to the population register, and those subjects were invited to participate by letter and by advertisements in local newspapers and public places. A prerequisite for eligibility was reading and writing skills in Swedish. The rationale for inviting women but not men born between 1945 and 1950 was to increase the sample size of young women to enable studies of breast cancer in premenopausal women.

30447 individuals attended the baseline examination, leading to a participation rate of 41%. Differences between participants and non-participant are previously described [113]. About 13% of those who participated were born outside Sweden. The majority had immigrated from Denmark, Yugoslavia, Poland, Germany, Finland and Hungary [112].

Baseline examination

Baseline examination included dietary assessment, blood pressure, height, and weight, a self-administered questionnaire, and collection of blood [112]. Blood components were separated, frozen and stored in the biological bank. In total, 1998 subjects failed to complete the baseline examination, i.e. the dietary assessment, the anthropometric measurements or the questionnaire, leaving 28449 individuals with complete data.

The baseline questionnaire, which was handed out at the first visit and checked for missing values at the second visit, included questions concerning education, occupation, physical activity, use of alcohol and tobacco, family history of disease, current health, medical condition and medication.

Dietary assessment was performed according to a modified diet history method described previously [114]. The participants completed a 168-item diet questionnaire concerning frequencies and portion sizes of regularly consumed foods, and a 7-day menu book that collected information on cooked meals, beverages (including alcohol), drugs and dietary

supplements. This was followed by a complementary 1-hour diet history interview. The validity of this method was tested [115].

The study protocols were approved by the ethics committee of Lund University. All participants provided written informed consent.

Definitions

DM at baseline was defined as self-report of a physician diagnosis or use of DM medication or fasting blood glucose (FBG) of ≥6.1 mmol/L (corresponding to fasting plasma glucose concentration of ≥7.0 mmol/L). Hyperinsulinemia was defined as the top quartile of fasting plasma insulin concentration in the segment of the population without DM, as proposed by the European Group for the study of Insulin Resistance [34]. Obesity was defined as BMI ≥30 kg/m^2. Waist circumference (waist) was measured as the circumference at the umbilicus at the end of a normal expiration and abdominal obesity was defined as waist >102 cm in men and >88 cm in women. Subjects who belonged to the top quartile of plasma CRP concentration were defined as having high CRP. Subjects were classified as having the MetS according to the NCEP ATP III criteria (Table 1). Blood pressure (BP) was measured using a mercury-column sphygmomanometer after ten minutes of rest in the supine position, and hypertension was defined as baseline systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg or use of anti-hypertensive treatment (AHT) according to the baseline questionnaire or the 7-day menu book. Prevalent cardiovascular disease was defined as occurrence of myocardial infarction or stroke prior to the baseline examination obtained through national registers as described previously [116]. Family history of DM was obtained by the baseline questionnaire and defined as known DM in at least 1 first-degree relative.

Leisure-time physical activity was assessed on the basis of a list of activities (18 items) adapted from the Minnesota Leisure Time Physical Activity instrument [117]. On the basis of information on physical activity provided from the self-administered baseline questionnaire described previously, a score was obtained by multiplying the reported amount of minutes per week spent on a specific activity by an activity-specific factor. According this score, the population was ranked into quartiles, and low physical activity was defined as the lowest quartile.

Socio-economic status was assessed on the basis of information on occupation provided from the baseline questionnaire [118]. Occupational status was based on questions concerning job titles and actual work tasks, and individuals were classified into one of five categories: high-level non-manual employees (e.g., business executives, engineers with a university degree, and university teachers), medium level non-manual employees

(e.g., registered nurses, computer operators, and high school teachers), low-level non-manual employees (e.g., office assistants, sales staff, and secretaries), skilled manual workers (e.g., vehicle mechanics, metal workers, and construction workers), and unskilled manual workers (e.g., factory workers, waiters, and cleaners). Based on these five categories a variable containing two groups was created, with high- and medium level non manual employees classified as high socio-economic status, and the other three categories classified as low socio-economic status. Homemakers together with farmers and owners of business enterprises were excluded from the analyses because of their unclear status in relation to the other groups.

Alcohol consumption was divided into four categories. Individuals with no consumption of alcohol in the menu book, and who indicated no consumption of alcohol during the previous year in the socioeconomic and lifestyle questionnaire, were categorised as zero consumers. The other subjects were categorized into three groups according to their alcohol consumption (for women <15 g alcohol per day was considered as low, 15–30 g as medium and >30 g high consumption; for men the corresponding figures were <20 g, 20-40 g and >40 g).

Cigarette smoking was elicited by a self-administered questionnaire, with current cigarette smoking defined as any smoking within the past year.

Malmö diet and cancer cardiovascular cohort
From MDC, a random 50% of those who entered the MDC study between November 1991 and February 1994 were invited to a sub-study for the epidemiology of carotid artery disease. This sample is referred to as the MDC cardiovascular cohort (MDC-CC) (Figure 5) and consists of 6103 individuals who underwent additional blood tests and an ultrasonographic examination of the carotid artery [116]. Of those, DNA is available in 6055 individuals.

In addition to the non-fasting baseline blood collection included in the MDC baseline investigation, fasting plasma samples were obtained at baseline in 5405 of the individuals included in MDC-CC. This additional blood testing in MDC-CC includes fasting whole blood glucose, HbA1c, insulin, total cholesterol, HDL and TG. LDL cholesterol was calculated using the friedewald formula. In retrospect, using stored plasma from baseline fasting samples, several peptide biomarkers recently was measured in MDC-CC including copeptin, mid-regional atrial natriuretic peptide, cystatin C and CRP. The plasma had been frozen at -80°C.
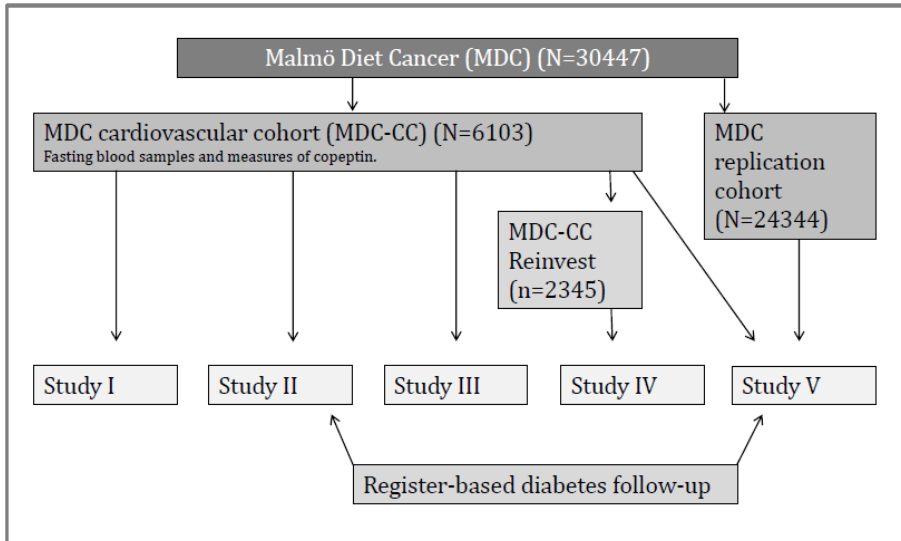
Figure 5: Description of study populations. The part of MDC that does not belong to MDC-CC we chose to name the MDC replication cohort as we use it for replication of genetical findings in Study V.

FOLLOW-UP WITH REGISTERS AND REINVESTIGATIONS

Register based diabetes diagnosis

The MDC population was followed up using six different national and regional DM registers.

In Study II, three registers were used to track new-onset DM diagnosed until December 2005: The Malmö HbA1c register (MHR), the nationwide Swedish National Diabetes Register (NDR) [119] and the regional Diabetes 2000 register of the Scania region, of which Malmö is the largest city [120]. NDR and Diabetes 2000 registers required a physician diagnosis according to established diagnostic criteria. The MHR analyzed and catalogued all HbA1c samples at the Department of Clinical Chemistry taken in institutional and non-institutional care in the greater Malmö area from 1988 onwards. Individuals who had at least two HbA1c recordings ≥6.0% in the MHR using the Swedish Mono-S standardization system (corresponding to 7.0% according to the US National Glycohemoglobin Standardization Program [NGSP]) were considered as having DM.

Individuals free of DM at baseline examination in MDC-CC (defined by lack of self-reported history of physician-diagnosed DM, use of DM medication, or FBG at the baseline examination of ≥6.1 mmol/L) and who were registered as DM cases any time after their baseline examination were classified as having new-onset DM (Study II).

In Study V, three other registers in addition to the three above mentioned registers were used to track DM diagnosed both before and after the baseline examination until July 2009 in the complete MDC cohort: the Swedish National Patient Register, which covers all somatic and psychiatric hospital discharges and Swedish hospital-based outpatient care [121], the Swedish Cause-of-Death Register [122] and the Swedish Prescribed Drug Register [123].

Reinvestigations and definitions

In MDC-CC, 2345 individuals had been reinvestigated from January 2007 to March 2010 (participation rate 70% of those invited) resulting in a mean follow-up time of 15.8 years. This cohort is called MDC-CC Reinvest (Figure 5) and was used for incidence analyses of MetS, abdominal obesity, obesity, hypertension and diabetes in Study IV. In the beginning of 2012, the complete MDC-CC had been reinvestigated (n=3734, participation rate 67%), and data on DM at follow-up from this complete reinvestigation was used in Study V. At the reinvestigation, a protocol similar to that applied at the baseline examination was used, but with additional measurement of

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urine albumin excretion and a 75 gram OGTT after an overnight fast, with measurement of plasma glucose at time 0 (before) and 120 min after glucose ingestion.

At the MDC-CC reinvestigation (Study IV), subjects free of DM at the baseline examination in the MDC-CC (defined by lack of self-reported history of physician-diagnosed DM, use of DM medication, or FBG at the baseline examination of ≥6.1 mmol/L) were classified as incident DM cases if they had a self-reported physician diagnosis, or if they used DM medication, or if they had a plasma glucose of ≥7.0 mmol/L or a 120 min value post OGTT plasma glucose >11.0 mmol/L.

The prevalence of hypertension at baseline was as high as 61.2% (Table 1). Instead of excluding 61.2% of the population in the incidence analysis of hypertension, we only excluded subjects on AHT at baseline and used initiation of AHT during follow-up as the endpoint, assuming that initiation AHT is a more valid indicator of a diagnosis of hypertension.

Microalbuminuria at reinvestigation was defined according to Swedish upper (95%) reference limits of ≥3.0 g albumin/mol creatinine in a morning urine sample [124].

Other definitions were the same at the reinvestigation as those at the baseline examination.

In addition to the above mentioned extensive reinvestigation, a subset (n=887) of the MDC-CC without known DM at baseline were selected for an extended study of factors associated with insulin resistance, estimated by the homeostatic assessment model of insulin resistance (HOMA-IR) index, and was reinvestigated after a mean of 6.6 years [125]. Fasting plasma glucose from this subset was used to validate the register-based DM findings in Study II.

As genetic exposure is constant throughout life, in Study V we classified participants as having DM regardless of whether DM was established before or at the baseline examination or during follow-up. Most DM diagnoses were captured using the 6 different registers described above. Some diagnoses were captured in the baseline questionnaire and yet some in the MDC-CC baseline investigation and in the MDC-CC reinvestigation. In addition, DM diagnoses were captured by fasting plasma glucose analyzed in a reinvestigation of about 1/3 of the MDC participants who also participated in the Malmö Preventive Project [126].

LABORATORY MEASUREMENTS

Plasma analyses

All analyses in plasma and whole blood were performed in overnight fasting samples. Analyses of fasting blood glucose, plasma lipids and insulin were carried out at the time of baseline examination at the Department of Clinical Chemistry, Skane University Hospital in Malmö, which is attached to a national standardization and quality control system. Fasting glucose was measured in whole blood by a hexokinase-glucose-6-phosphate dehydrogenase method. In fasting plasma samples, copeptin was measured using a commercially available sandwich eliza assay in the chemiluminescence/coated tube format (B.R.A.H.M.S AG, Hennigsdorf, Germany) as described previously [127]. CRP was measured by a high-sensitivity assay (Tina-quant CRP, Roche Diagnostics, Basel, Switzerland). Cystatin C was measured using a particle-enhanced immune nephelometric assay (N Latex Cystatin C, Dade Behring, IL). LDL cholesterol values were estimated according to the Friedewald's formula [128]. HOMA-IR values were calculated as (plasma insulin x plasma glucose)/22.5 [129]. Albumin and creatinine were measured in morning urine samples with methods described earlier [124].

Genotyping

DNA was extracted from frozen granulocyte or buffy coat samples collected from MDC-CC (Study I) and from the complete MDC cohort (Study V) with the use of QIAamp-96 spin blood kits (QIAGEN, Stockholm, Sweden) at the DNA extraction facility supported by SWEGENE. To analyze the polymorphisms of AVPR1A (Study I) and AVPR1B (Study V) and capture the maximum of the genetic variance of the genes, data from HapMap were used (www.hapmap.org) to select tag SNPs. In the AVPR1A gene, rs1042615, rs10747983, rs10784339, and rs7308855 were selected, and in the AVPR1B gene, rs35810727, rs28373064, rs35439639, and rs35608965 were selected. Primers and probes were custom synthesized by Applied Biosystems (Foster City, CA) according to standard recommendations for the AB Prism 7900HT analysis system, and genotyped with polymerase chain reaction-based methods as previously described [130].

STATISTICS

SPSS statistical software (version 14.0, 17.0 and 20.0) was used for all analyses but calculation of the C statistics (Study II), which was performed using Stata software version 8.0 (stata corp).

Continuous variables which were not normally distributed (glucose, TG, insulin, CRP and copeptin) were transformed using the natural logarithm before statistical analyses were performed (Study I-V). However, when glucose was analyzed in a non-diabetic population (Study II), it was normally distributed, why it was not necessary with logarithmic transformation.

Group-wise differences in continuous variables at baseline were tested using student's t-test or ANOVA and reported as means ± SD if normally distributed and with Mann-Whitney test and reported as medians and interquartile ranges if not normally distributed. Differences in dichotomous variables were tested using chi-square tests.

Multivariate adjusted logistic (for dichotomous outcome variable) and linear regression (for continuous outcome variable) models were used to test the relationship between quartiles of copeptin and the outcome variable (Study II-IV), or to test the relationship between genotype and the outcome variable (Study I and V). A two-sided P-value <0.05 was considered statistically significant.

In Study II, we stratified the cohort according to copeptin level into four quartiles. As copeptin is known to be significantly higher in men than women, we adjusted for sex in all analyses. In Study III and IV, copeptin quartiles which were pooled according to sex were used in all analyses. This was done to mitigate the occurrence of men in the top quartile of copeptin. In incidence analyses of MetS, abdominal obesity, obesity, hypertension and DM (Study II and IV), we excluded subjects with baseline prevalence of the outcome variable in question.

In the quartile analyses, the relationship between copeptin levels and the outcome variable was expressed as OR for each quartile with the lowest quartile defined as the referent (OR=1.0), and as OR per quartile increase to obtain the P trend over quartiles.

Analyses of dietary fat intake

Dietary fat intake in relation to total energy intake was calculated (Study I and III). After logarithmic transformation, we regressed total fat intake (in grams) on total energy intake in males and females separately. The residuals were saved and used to rank individuals. In Study I the study population was stratified into sex-specific quartiles of relative intake of dietary fat. In Study III, a sex-specific dichotomous variable with above and below median of relative intake of fat was used. By using residuals of fat intake in relation to total energy intake instead of total fat intake to rank individuals, we reduced confounding from dietary over- and underreporting of energy. Because dietary patterns and self-reported dietary intakes tend to differ according to sex [131, 132], analyses that included fat intake were analyzed in male and female subjects separately (Study I and III). Furthermore, all analyses that included measures of fat intake (Study I and III) were adjusted for physical activity.

As a diagnosis of DM usually leads to a change in dietary pattern, subjects with known DM (i.e. reporting a history of DM or being under treatment with antidiabetic agents) were excluded from the analyses concerning fat intake. Thus, in these analyses, all DM cases were new onset diabetes cases defined solely by having FBG $\geq$6.1 mmol/L on baseline screening. In Study I, we tested the association between genetic variance in rs1042615 and TG in strata of fat intake. As DM was associated with rs1042615 among men with high fat intake, and DM is known to be associated with elevated TG levels, these analyses were performed among non-DM subjects only.

Discrimination

To assess the relationship between sensitivity and specificity of copeptin in predicting new-onset DM in addition to classical DM predictors (Study II), we compared the area under the Receiver Operating Characteristic (ROC) curves using both a personal model (age, gender, BMI and family history of DM) and a clinical model (personal model + systolic blood pressure, TG, HDL, waist and FBG) as previously proposed [133], with and without copeptin in each of the two models. The Integrated Discrimination Improvement (IDI) was calculated as described previously [134]. In addition to logistic regression we performed Cox regression models with the time of the DM event defined as the date of either the first HbA1c value $\geq$6% in MHR, registration in DM 2000, or registration in NDR (Study II). C statistics based on the Cox regressions were calculated as described previously [135].

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RESULTS

CHARACTERISTICS AT BASELINE AND FOLLOW-UP

Baseline characteristics for the MDC-CC are shown in Table 2. The table includes 4742 individuals as complete fasting plasma samples were available in 5405 MDC-CC participants, and data on covariates including components of the MetS, potential confounders, and copeptin were available in 4742 of those participants. Medians (interquartile range) of copeptin (in pmol/l) in quartiles 1 to 4 were in men: 3.16 (2.21-3.80), 5.56 (4.86-6.27), 8.44 (7.64-9.53), 13.5 (11.50-16.60); and in women: 1.86 (1.36-2.34), 3.41 (3.04-3.77), 5.14 (4.70-5.76), 8.41 (7.22-10.45).

Table 2. Population-description of MDC-CC (n=4742)			
	Men+Women	**Men**	**Women**
Age (years)	57.5 ± 5.9 [a]	57.7 ± 6.0	57.4 ± 5.9
Sex (% men)	40.5	NA	NA
Copeptin (pmol/l) [b]	5.14(3.20-8.15)	7.07(4.57-10.60)	4.23(2.70-6.42)
Hypertension (%)	63.3	68.2	60.0
AHT (%)	16.5	17.8	15.6
CRP (mg/l) [b]	1.3 (0.7-2.8)	1.4 (0.7-2.8)	1.3 (0.7-2.8)
HDL (mmol/l)	1.39 ± 0.37	1.21 ± 0.30	1.51 ± 0.37
TG (mmol/l) [b]	1.14 (0.86-1.58)	1.27 (0.95-1.74)	1.07 (0.81-1.47)
Waist (cm)	83.5 ± 12.7	93.0 ± 9.9	77.0 ± 10.1
Abdominal obesity (%)	14.0	15.1	13.2
BMI (kg/m²)	25.7	26.1 ± 3.4	25.4 ± 4.1
Obesity (%)	12.5	11.4	13.2
FBG (mmol/l)	5.13 ± 1.27	5.34 ± 1.44	4.99 ± 1.12
Diabetes (%)	7.7	10.7	5.7
MetS (%)	21.5	25.6	18.7
Insulin (mU/L) [b]	6.0 (4.0-9.0)	7.0 (5.0-10.0)	6.0 (4.0-9.0)
Smoking (%)	25.6	26.5	25.1
Diabetes heredity (%)	3.0	2.0	3.7
History of CVD (%)	2.2	3.8	1.1
Abbreviations: AHT, antihypertensive treatment; BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; FBG, fasting blood glucose; HDL, high density lipoprotein cholesterol; MetS, metabolic syndrome; TG, triglycerides; waist, waist circumference. [a] Mean ± SD (all such values if nothing else specified). [b] Expressed as median (interquartile range).			

Diabetes

In MDC-CC, a total of 365 individuals had DM at baseline according to self-report of physician DM diagnosis, use of DM medication, or FBG at the baseline examination of ≥6.1 mmol/L (Study II). Of those, 29% had a history of a physician-diagnosis of DM or DM treatment and 71% had an FBG ≥6.1 mmol/L without an accompanying physician diagnosis. Among participants free of DM at baseline (n=4377), 174 subjects developed new-onset DM according to the MHR, NDR and Diabetes 2000 registers during a mean follow-up time of 12.6 years (Study II). There was a considerable overlap between the different registers. MHR captured 106 cases of DM, whereas the Diabetes 2000 registry and NDR captured 110 and 76 cases, respectively. When the baseline sample was further restricted to subjects without IFG (n=3702), 79 subjects developed new-onset DM during follow-up. Among the subjects in MDC-CC that were reinvestigated between January 2007 and March 2010 (n=2345, complete data on n=2064), 308 subjects (16.0%) developed new-onset DM during the average follow-up time of 15.8 years when DM was defined as either self-report of a physician diagnosis or use of DM medication after the baseline examination, or FPG of ≥7.0 mmol/L or 120 min value post OGTT plasma glucose >11.0 mmol/L (Study IV).

In the complete MDC cohort, 1588 individuals (DNA available in n=1517) had DM at baseline (Study V) according to registers and self-report of physician DM diagnosis, use of DM medication, or (which was only available in the MDC-CC) FBG of ≥6.1 mmol/L. 3067 individuals (DNA available in n=2913) developed DM during a mean follow-up time of 14.0 years according to 6 registers and findings at reinvestigation (Study V).

The metabolic syndrome and its components

The prevalence of MetS in MDC-CC was 21.5% and higher in men than in women (Study III). The prevalences of the individual components included in MetS are found in Table 2. Among the subjects in MDC-CC that were reinvestigated between January 2007 and March 2010 in Study IV (n=2345, complete data on n=2064), 433 subjects (26.2%) developed MetS, 236 (12.9%) developed obesity, 533 (29.6%) developed abdominal obesity and 730 (42.2%) started AHT treatment. The prevalence of microalbuminuria at baseline investigation was not measured, but the prevalence at reinvestigation was 191 (9.3%) among subjects with DM at baseline and 159 (8.2%) among subjects without DM at baseline (Study IV).

COPEPTIN: CROSS-SECTIONAL FINDINGS

In Study II, we found that elevated copeptin was cross-sectionally associated with DM after multivariable adjustment for all baseline covariates that differed between DM and subjects without DM (i.e. age, sex, HDL, TG, blood pressure, AHT, BMI, waist, waist-to-hip ratio, cystatin C, CRP, and history of CVD) except for FBG and insulin level. After this adjustment, the odds ratio (OR) for DM increased in quartile Q2–Q4 of copeptin compared with the lowest quartile (Q1) of copeptin [Q2: 1.19, 95% confidence interval (CI) 0.81–1.75; Q3: 1.39, 0.96–2.01 and Q4: 1.45, 1.00–2.11; P trend 0.04] (Study II). Furthermore, the OR for hyperinsulinemia increased in Q2-Q4 of copeptin compared with Q1 after multivariable adjustment for age, sex, HDL, TG, blood pressure, AHT, BMI, waist, waist-to-hip ratio, cystatin C, CRP, history of CVD and FBG [Q2: 1.19, 95% CI 0.94–1.51; Q3: 1.25, 0.99–1.59 and Q4: 1.61, 1.26–2.06; P trend <0.001]. In the segment of the population free of DM, copeptin was positively associated with FBG and P-Insulin (Figure 6).
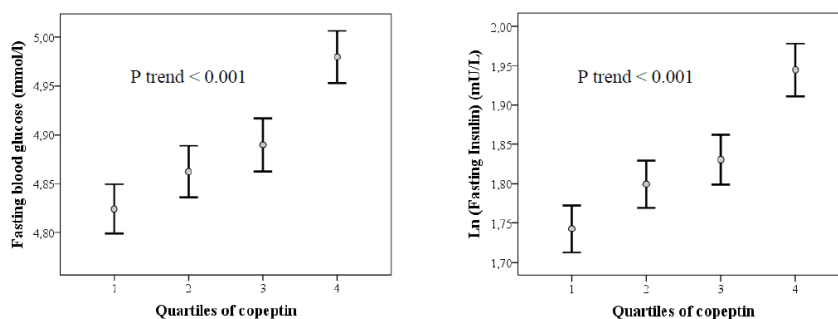
Figure 6. Fasting concentrations of glucose in whole blood and insulin in plasma (the latter logarithmically transformed), expressed as mean (95% CI), in non-diabetic subjects stratified into quartiles of increasing copeptin (n=4377).

In Study III we found that increasing copeptin quartile was cross-sectionally and positively associated with MetS after adjustment for age and sex, and with hypertension, waist, BMI and CRP, but not with TG and HDL, after adjustment for age and sex, insulin and DM (Table 3). After additional adjustment for cystatin C, we found that hypertension (P trend=0.02), CRP (P trend=0.001), and waist (P trend=0.04) remained significantly associated with copeptin, whereas BMI (P trend=0.14) did not. In the next step, we tested whether the MetS components were associated with copeptin independently of each other. We used models which in

addition to age, sex, DM and insulin were adjusted for all other MetS variables that were significantly associated with copeptin (Table 3). Increasing copeptin quartile remained significantly associated with hypertension (P trend=0.01), high CRP (P trend=0.04) and abdominal obesity (P trend=0.02), and borderline significantly associated with obesity (P trend=0.09).

Copeptin, the metabolic syndrome and environmental factors

High levels of copeptin were associated with high fat intake (P<0.001) and low physical activity (P=0.01), and showed a borderline significant association with low socioeconomic status (P=0.09) in linear regression models adjusted for age and sex, and additionally adjusted for physical activity in analyses concerning fat intake (Study III). We did not find any significant associations between levels of copeptin and smoking or alcohol consumption. The association between copeptin and fat intake remained significant when the association was analyzed in men (P=0.02) and women (P=0.003) separately, whereas the association with physical activity remained significant among men (P=0.02) but not among women (P=0.13). Furthermore, we investigated whether the association between quartiles of copeptin and components of the MetS were independent from the environmental factors fat intake, socioeconomic status and physical activity. We found that copeptin remained cross-sectionally associated with hypertension (P trend<0.001), CRP (P trend<0.001), waist (P trend<0.001), BMI (P trend<0.001), DM (P trend=0.005), hyperinsulinemia (P trend<0.001) and MetS (P trend<0.001) independently of age, sex, fat intake, socioeconomic status and physical activity (Study III). Furthermore, because dietary patterns and self-reported dietary intakes tend to differ according to sex, analyses that included fat intake were analyzed in male and female subjects separately. In addition, copeptin is known to be higher among men, and the impact of environmental factors on the observed associations between copeptin and MetS components may differ between men and women. Thus, we analyzed men and women separately to evaluate whether the associations between copeptin quartiles and components of MetS were independent of environmental factors in both men and women. These associations remained significant in men and significant or borderline significant in women (data not shown).

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Table 3. Baseline plasma copeptin quartiles (Q1-Q4) in relation to components of the metabolic syndrome – cross-sectional findings.			P trend[a]
Waist circumference (Mean ± SD)	Q1	82.0 ±12	0.007
	Q2	83.4 ± 12	
	Q3	83.8 ± 13	
	Q4	84.9 ± 13	
Body mass index (Mean ± SD)	Q1	25.1 ± 3.6	0.03
	Q2	25.8 ± 3.8	
	Q3	25.9 ± 3.7	
	Q4	26.1 ± 4.3	
High density lipoprotein (Mean ± SD)	Q1	1.42 ± 0.37	0.39
	Q2	1.39 ± 0.37	
	Q3	1.38 ± 0.38	
	Q4	1.37 ± 0.37	
Triglycerides (Median (interquartile range))	Q1	1.09 (0.82-1.46)	0.10
	Q2	1.14 (0.85-1.58)	
	Q3	1.15 (0.87-1.63)	
	Q4	1.21 (0.90-1.64)	
C-reactive protein (Median (interquartile range))	Q1	1.1 (0.6-2.4)	<0.001
	Q2	1.3 (0.6-2.7)	
	Q3	1.4 (0.7-2.8)	
	Q4	1.6 (0.8-3.2)	
Hypertension (%)	Q1	58.8	0.004
	Q2	61.5	
	Q3	63.9	
	Q4	69.4	
Metabolic syndrome (%)	Q1	15.0	<0.001[b]
	Q2	21.5	
	Q3	24.3	
	Q4	25.5	

[a] P value in logistic or linear regression adjusted for age, sex, DM and insulin.
[b] Adjusted only for age and sex.

Copeptin and the prediction of diabetes

The likelihood of developing DM increased with increasing copeptin quartiles after multivariate adjustment in both subjects free of DM at baseline and subjects free of IFG at baseline (Table 4).

We evaluated the discriminative ability of copeptin in DM prediction using ROC curves and IDI. In subjects free of DM at baseline, the area under the ROC curve increased from 0.694 to 0.710 (P=0.08) when copeptin was added to the personal model, and from 0.832 to 0.841 (P=0.007) when copeptin was added to the clinical model of DM prediction. In subjects free of IFG at baseline, the area under the ROC curve increased from 0.663 to 0.713 (P=0.03) and from 0.783 to 0.805 (P=0.04) when copeptin was added to the personal model and clinical model for DM prediction, respectively. IDI was significantly improved by adding copeptin to the personal model for DM prediction in both non-DM subjects (P=0.01) and non-IFG subjects (P=0.02) but non-significant when added to the clinical model (P=0.35 in nondiabetic individuals, P=0.09 in non-IFG individuals).

Table 4. Baseline plasma copeptin quartiles (Q1-Q4) in relation to incident diabetes after 12.6 years of register based follow-up.			
		OR (95% CI)	P trend
Incident DM among non-DM at baseline [1]	Q1	1.00 (ref)	0.004
	Q2	1.37 (0.78-2.39)	
	Q3	1.79 (1.06-3.05)	
	Q4	2.09 (1.23-3.56)	
Incident DM among non-IFG at baseline [2]	Q1	1.00 (ref)	0.001
	Q2	1.80 (0.78-4.16)	
	Q3	1.92 (0.84-4.38)	
	Q4	3.48 (1.58-7.65)	

Abbreviations: DM, diabetes mellitus; IFG, impaired fasting glucose
[1] Subjects who developed DM during follow-up (n=174) among subjects without DM at baseline (n=4377).
[2] Subjects who developed DM during follow-up (n=79) among subjects without IFG at baseline (n=3702).
Models adjusted for age, sex, high density lipoprotein, triglycerides, blood pressure, antihypertensive treatment, body mass index, waist circumference, waist-to-hip ratio, cystatin C, C-reactive protein, prevalent cardiovascular disease, smoking, family history of DM, low density lipoprotein, fasting blood glucose and fasting insulin.

C statistics derived from Cox regression models were similar to the ROC-curves derived from logistic regression models. With the addition of copeptin, the C statistic from Cox regression models increased in subjects without DM at baseline from 0.702 to 0.718 in the personal model and from 0.835 to 0.844 in the clinical model. When analyses were restricted to subjects without IFG at baseline, the C statistic increased from 0.671 to 0.721 in the personal model and from 0.790 to 0.811 in the clinical model.

Validation of longitudinal diabetes findings
In a subset of the MDC-CC that was reinvestigated after 6.6 years (n=887), we investigated the association between quartiles of copeptin and DM development (diagnosed on the basis of elevated FBG) in order to validate our register-based DM findings (Study II). Among those free of DM at baseline, 63 subjects were diagnosed as DM cases at follow-up. After full adjustment (corresponding to the adjustment used in Table 4) increasing quartile of copeptin was associated with new-onset DM both in subjects free of DM at baseline (OR, 1.42; 95% CI, 1.04 to 1.94; P=0.03) and in subjects free of IFG at baseline (OR, 1.67; 95% CI, 1.07 to 2.63; P=0.03), comparable to the findings reported in Table 4.

In Study IV, the predictive value of copeptin in DM development was further validated when the DM incidence was assessed using data from the MDC-CC reinvestigation (15.8 years of follow-up) instead of the register based follow-up. We found that DM at reinvestigation was significantly associated with increasing copeptin quartile (Table 5, Figure 7).

Copeptin and incident metabolic syndrome
Using data from the MDC-CC reinvestigation (15.8 years of follow-up) copeptin was not found to be an independent predictor of the cluster of MetS at reinvestigation (Table 5). Neither did we find any association between copeptin quartile and the incidence of obesity or use of anti-hypertensive treatment (Table 5) (Study IV).

Copeptin and the prediction of abdominal obesity
Increasing quartile of baseline copeptin predicted incident abdominal obesity at the MDC-CC reinvestigation after 15.8 years of follow-up after multivariate adjustment (Table 5, Figure 7) (Study IV).

As increasing quartile of copeptin predicted both incident abdominal obesity and incident DM after multivariate adjustment (Table 5), the model

was further adjusted for incident DM on top of adjustment for age, sex, hypertension, glucose, TG, HDL, waist, cystatin C and follow-up time, and the association between copeptin and incident abdominal obesity remained significant [odds ratio (95% confidence interval) in quartiles 1 to 4, respectively: 1.00 (reference), 1.55 (1.08-2.21), 1.30 (0.91-1.85), 1.60 (1.12-2.29); P trend=0.03]. Similarly, we adjusted the relationship between baseline copeptin and incident DM for incident abdominal obesity on top of the multivariate adjustment, and also this relationship remained significant [odds ratio (95% confidence interval) 1.00 (reference), 1.17 (0.78-1.74), 1.31 (0.88-1.94), 1.45 (0.98-2.14); P trend=0.049].
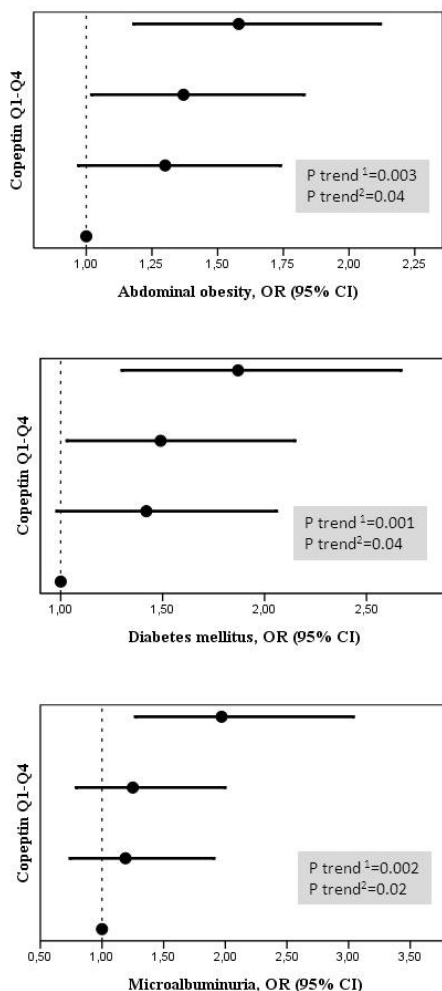

Figure 7. Baseline plasma copeptin quartiles (Q1-Q4) in relation to incidence of abdominal obesity, diabetes and microalbuminuria at reinvestigation.
[1] Model adjusted for follow-up time, age and sex.
[2] Model adjusted for follow-up time, age, sex, cystatin C, hypertension, glucose, triglycerides, HDL and waist circumference.

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Table 5. Baseline plasma copeptin quartiles (Q1-Q4) in relation to incidence of components of the metabolic syndrome and microalbuminuria at reinvestigation after 15.8 years of follow-up.

		OR (95% CI)	P trend
Abdominal obesity, subjects without abdominal obesity at baseline **N=1799**	Q1	1.00 (ref)	0.04
	Q2	1.55 (1.08-2.21)	
	Q3	1.30 (0.91-1.84)	
	Q4	1.59 (1.11-2.28)	
Obesity, subjects without obesity at baseline **N=1832**	Q1	1.00 (ref)	0.86
	Q2	1.17 (0.76-1.80)	
	Q3	1.13 (0.74-1.73)	
	Q4	1.06 (0.69-1.63)	
Incident AHT, subjects without AHT at baseline **N=1731**	Q1	1.00 (ref)	0.45
	Q2	1.06 (0.79-1.43)	
	Q3	1.00 (0.74-1.34)	
	Q4	0.90 (0.66-1.22)	
Diabetes mellitus, subjects without diabetes at baseline **N=1928**	Q1	1.00 (ref)	0.04
	Q2	1.18 (0.79-1.76)	
	Q3	1.32 (0.89-1.96)	
	Q4	1.46 (0.99-2.16)	
Metabolic syndrome, subjects without metabolic syndrome at baseline **N=1653**	Q1	1.00 (ref)	0.19
	Q2	1.21 (0.85-1.72)	
	Q3	1.05 (0.74-1.49)	
	Q4	1.34 (0.95-1.91)	
Microalbuminuria, subjects with and without diabetes at baseline **N=2064**	Q1	1.00 (ref)	0.02
	Q2	1.05 (0.65-1.69)	
	Q3	1.08 (0.67-1.73)	
	Q4	1.65 (1.06-2.59)	
Microalbuminuria, subjects without diabetes at baseline **N=1928**	Q1	1.00 (ref)	0.02
	Q2	1.14 (0.69-1.91)	
	Q3	1.01 (0.60-1.70)	
	Q4	1.78 (1.11-2.88)	

Abbreviations: AHT, antihypertensive treatment.
Models adjusted for follow-up time, age, sex, cystatin C, hypertension (systolic and diastolic blood pressure in analyses with incident AHT as outcome), glucose, triglycerides, high density lipoprotein and waist circumference.
Subjects with baseline prevalence of the outcome variable were excluded from the analyses.

Copeptin and microalbuminuria

Increasing quartiles of copeptin at baseline was associated with microalbuminuria at reinvestigation after multivariate adjustment both before and after exclusion of prevalent DM from the cohort (Table 5, Figure 7). As there is a strong link between CRP and microalbuminuria [136], we further adjusted for baseline CRP on top of the multivariate adjustment, and found that the association across increasing copeptin quartiles (Q1 as reference) among subjects free from DM at baseline, was not affected [Q2: OR 1.14, 95% CI 0.68-1.91; Q3: 1.03, 0.61-1.73 and Q4: 1.91, 1.18-3.09; P trend=0.01]. Finally, on top multivariate adjustment and CRP, we additionally adjusted for incident hypertension and incident DM, and found that the association across increasing quartiles of copeptin (Q1 as reference) remained significant [Q2: OR 1.09, 95% CI 0.65-1.83; Q3: 0.97, 0.57-1.66 and Q4: 1.82, 1.12-2.96; P trend=0.02].

GENETIC VARIANCE IN VASOPRESSIN RECEPTORS

Genetic variance in AVPR1A and phenotypic resemblance to V1aR deficient mice

In Study I, we investigated tag SNPs of the human AVPR1A gene in relation to metabolic phenotype in MDC-CC and found that carriers of at least one rs1042615 T-allele had significantly lower levels of TG (Table 6). After adjustment for age, gender, physical activity and BMI, the association was still significant (P=0.025). Further inclusion of glucose in the model strengthened the association even more (P=0.001), and when analysing the association among individuals without DM, we found that T-allele carriers had highly significantly lower TG than carriers of the CC genotype (P=0.001). None of the other tag SNPs (rs10747983, rs10784339, rs7308855) were significantly associated with altered TG levels (data not shown).

Furthermore, individuals carrying one or two rs1042615 T-alleles had significantly higher FBG than carriers of the CC-genotype (Table 6). The association between the T-allele and elevated FBG remained significant after adjustment for age, gender, physical activity and BMI (P=0.036) and was strengthened after additional inclusion of TG in the model (P=0.005). The OR for DM in carriers of the T-allele was 1.22; 95% CI 0.99-1.51; P=0.067. AVPR1A tag SNPs rs10747983, rs10784339 and rs7308855 were neither significantly associated with FBG level nor with DM status.

Table 6 – Variation in V1aR rs1042615 in relation to metabolic phenotype.
N=5506, genotyping success rate 96.8%.

Genotype	**CC** (30.4%)	**CT** (49.2%)	**TT** (20.4%)	P-value[1]	P-value[2]
Triglycerides (mmol/l)	1.42 ± 0.89	1.36 ± 0.79	1.36 ± 0.71	0.037	0.014
Glucose (mmol/l)	5.12 ± 1.22	5.21 ± 1.45	5.19 ± 1.44	0.11	0.036
Diabetes (%)	7.7	9.7	8.1	0.052	0.067
Obesity (%)[3]	12.5	13.2	15.1	0.12	0.19
BMI (kg/m²)[3]	25.9 ± 3.8	25.7 ± 4.0	26.0 ± 4.1	0.10	0.74

Data are expressed as mean $\pm$ SD.
[1] ANOVA (for triglycerides, glucose and BMI), Chi-square test (for diabetes and obesity).
[2] CC vs CT/TT genotype: T-test (for triglycerides, glucose and BMI), Logistic Regression (for diabetes and obesity).
[3] n=6055

The finding that the phenotype of human carriers of the rs1042615 T-allele (increased FBG and decreased TG levels) resembled that of the V1aR deficient knockout mice [69, 84] encouraged us to test for further phenotypic similarities. As male V1aR deficient mice were more prone than the wild type mice to develop overt DM and obesity when fed a high fat diet, we tested the rs1042615 T-allele for phenotypic association within gender specific strata of fat intake ($Q1_{FAT}$-$Q4_{FAT}$). As known DM commonly leads to changes in dietary patterns, we excluded patients with a previous diagnosis of DM (79 men and 76 women).

Among men with the highest fat intake ($Q4_{FAT}$) the rs1042615 T-allele was significantly associated with DM when the model was adjusted for age, BMI and physical activity [OR 2.22, 95% CI 1.05-4.71, P=0.03], whereas there was no significant association in men belonging to $Q1_{FAT}$-$Q3_{FAT}$. The interaction term (fat intake x genotype) was significantly associated with DM in males (P=0.04). Among women, there was no association between the T-allele and DM in any of the quartiles of fat intake (data not shown). In line with these data, FBG levels were slightly higher in men within $Q4_{FAT}$ carrying the T-allele compared with carriers of the CC-genotype after adjustment for age, BMI and physical activity (5.27 ± 0.89 mmol/L versus 5.13 ± 0.57 mmol/L, P=0.061), whereas FBG levels did not differ between carriers and non-carriers of the T-allele among $Q1_{FAT}$-$Q3_{FAT}$.

We further tested whether the rs1042615 T-allele association to DM was affected by stratification for BMI ($Q1_{BMI}$-$Q4_{BMI}$). Similar to the association between the rs1042615 T-allele and DM within $Q4_{FAT}$ in men, the association between the T-allele and DM within $Q4_{BMI}$ was significant in men [OR 1.81, 95% CI 1.11-2.93, P=0.01]. The interaction term (BMI x genotype) was borderline significantly associated with DM in males (P=0.093). In concert with this finding, the T-allele was in men associated with significantly higher FBG within $Q4_{BMI}$ compared with carriers of the CC-genotype after adjustment for age, BMI and physical activity [6.04 ± 2.14 mmol/L versus 5.54 ± 1.47 mmol/L, P=0.006] whereas there was no significant difference according to genotype within any other quartile of BMI. In women, carrying of the T-allele was neither associated with a higher prevalence of DM nor an altered glucose level in any of the BMI quartiles. Using clinical cut-off levels in the entire sample of men for normal weight (n=871), overweight (n=1407) and obesity (n=283) showed that OR for DM in men carrying the T-allele compared to carriers of the CC-genotype was 0.88 (95% CI 0.48-1.62; P=0.69) among normal weight subjects, 1.51 (95% CI 1.08-2.13; P=0.02) among overweight subjects, and 1.71 (95% CI 0.89-3.31; P=0.11) among the quite small sample of obese men.

Finally, we tested whether the finding of relatively lower TG levels in carriers of the rs1042615 T-allele as compared to carriers of the CC-genotype was dependent on strata of fat intake or BMI. These analyses were performed in individuals without DM as DM per se is strongly associated with elevated TG levels, and as the rs1042615 T-allele was associated with DM in men within $Q4_{FAT}$. There was no consistent association between the T-allele and low TG-levels in any of the $Q1_{FAT}$-$Q4_{FAT}$ after adjustment for age, BMI and physical activity among either men or women. Finally, there was no association between the rs1042615 T-allele and TG among either men or women in the upper quartile of BMI.

Genetic variance in AVPR1B in body weight gain and diabetes

In MDC-CC, the major allele of the tag SNP rs35810727 in the AVPR1B gene was positively associated with BMI and overweight after adjustment for age and gender, whereas we did not find any significant association with obesity (Table 7a). Furthermore, the same polymorphism was associated with elevated waist after adjustment for age and gender, but we did not find any association with abdominal obesity (Table 7a). The association with BMI and overweight was replicated in the MDC replication cohort (Table 7b), whereas the association with waist was not significant in the MDC replication cohort (Table 7b). The AVPR1B tag SNPs rs28373064, rs35439639, rs35608965 were not associated with any measures of obesity (data not shown).

We did not find any association between genetic variance in AVPR1B and DM in the MDC-CC (Table 7a). However, the major allele of rs35810727 was positively associated with DM in the MDC replication cohort (Table 7b) and in the complete MDC cohort (n=30 447, OR 1.10, 95% CI 1.00-1.20, P=0.04) after adjustment for age and gender. When BMI was added to the model, the association between DM and rs35810727 remained in the MDC replication cohort (OR 1.15, 95% CI 1.03-1.29, P=0.01) but not in the complete MDC cohort (OR 1.07, 95% CI 0.97-1.17, P=0.18). The AVPR1B tag SNPs rs28373064, rs35439639 and rs35608965 were not significantly associated with DM (data not shown).

Copeptin levels and genetic variance in AVPR1A and AVPR1B

We tested the association between copeptin and genetic variation in AVPR1A (unpublished results) and AVPR1B (Study V) in the MDC-CC subset and found that none of the tag SNPs were associated with altered copeptin level (Table 8). When the associations between copeptin level and genetic variation were stratified by gender, we found that male T-allele

carriers of the rs1042615 had significantly lower copeptin levels than the CC genotype (Table 8).

Table 7a. Malmö diet and cancer cardiovascular cohort: Genetic variation of AVPR1B rs 35810727 in relation to metabolic phenotype.
N=6027, genotyping success rate 97.9%.

Genotype	AA (0.7%)	AC (13.1%)	CC (86.2%)	P-value[1]	Effect estimate[2]
BMI	25.4 ± 4.8	25.4 ± 4.0	25.8 ± 4.0	0.03	0.30 ± 0.14
Overweight	46.2	47.7	53.7	0.004	1.23 (1.07-1.41)
Obesity	15.4	12.0	13.6	0.37	1.10 (0.89-1.36)
Waist	82.3 ± 13.7	83.0 ± 12.7	84.4 ± 13.0	0.03	0.78 ± 0.36
Abdominal obesity	17.9	14.2	15.2	0.74	1.03 (0.85-1.26)
Diabetes	20.5	19.9	18.0	0.12	0.87 (0.73-1.04)

Continuous variables are given as means ± SD.
[1] Linear regression for continuous variables and logistic regression for dichotomous variables using additive model for the genetic effect. Models adjusted for age and sex.
[2] Beta-coefficient ± standard error for continuous variables, OR (95% CI) for dichotomous variables in models adjusted for age and sex.

Table 7b. Malmö diet and cancer replication cohort: Genetic variation of AVPR1B rs 35810727 in relation to metabolic phenotype.
N=22740, genotyping success rate: 95.4%

Genotype	AA (0.6%)	AC (14.3%)	CC (85.1%)	P-value[1]	Effect estimate[2]
BMI	25.6 ± 3.8	25.7 ± 4.0	25.9 ± 4.1	0.003	0.21 ± 0.07
Overweight	50.4	51.5	54.1	0.001	1.13 (1.05-1.21)
Obesity	13.3	13.4	14.0	0.30	1.06 (0.95-1.17)
Waist	84.1 ± 11.3	84.5 ± 19.1	84.4 ± 15.2	0.25	0.27 ± 0.007
Abdominal obesity	14.1	15.6	16.9	0.03	1.12 (1.01-1.23)
Diabetes	14.7	12.1	14.2	0.002	1.18 (1.06-1.32)

Continuous variables are given as means ± SD.
[1] Linear regression for continuous variables and logistic regression for dichotomous variables using additive model for the genetic effect. Models adjusted for age and sex.
[2] Beta-coefficient ± standard error for continuous variables, OR (95% CI) for dichotomous variables in models adjusted for age and sex.

Table 8. Copeptin in relation to AVP receptor polymorphism.

	β-coefficient (95% CI)	P-value
men and women		
AVPR1A		
rs1042615[a]	-0.03 (-0.09–0.03)	0.32
rs10747983	0.006 (-0.05–0.06)	0.81
rs10784339	0.008 (-0.04–0.06)	0.76
rs7308855	0.03 (-0.03–1.00)	0.35
AVPR1B		
rs35810727[b]	-0.01 (-0.09–0.06)	0.72
rs28373064	0.004 (-0.05–0.06)	0.87
rs35439639	-0.02 (-0.17–0.12)	0.74
rs35608965	-0.01 (-0.10–0.07)	0.76
men		
AVPR1A		
rs1042615[a]	-0.10(-0.17–(-0.02))	0.01
rs10747983	0.03 (-0.03–0.10)	0.32
rs10784339	0.03 (-0.04–0.09)	0.40
rs7308855	0.05 (-0.03–0.13)	0.22
AVPR1B		
rs35810727[b]	-0.02 (-0.12–0.07)	0.62
rs28373064	0.002 (-0.06–0.07)	0.95
rs35439639	-0.01 (-0.20–0.17)	0.89
rs35608965	-0.01 (-0.12–0.10)	0.85
women		
AVPR1A		
rs1042615[a]	0.02 (-0.07–0.10)	0.68
rs10747983	-0.01 (-0.09–0.06)	0.72
rs10784339	-0.007 (-0.08–0.07)	0.86
rs7308855	0.02 (-0.08–0.11)	0.75
AVPR1B		
rs35810727[b]	-0.006 (-0.11–0.09)	0.91
rs28373064	0.005 (-0.07–0.08)	0.88
rs35439639	-0.03 (-0.24–0.17)	0.77
rs35608965	-0.01 (-0.13–0.11)	0.81

N=5195. Models adjusted for age (and sex in analyses with both men and women). Values are given as β-coefficient (95% CI) expressed as number of SD increase of LN-transformed plasma copeptin concentration in relation to genotype. Minor allele was coded in additive models if nothing else specified.
[a] CC vs CT+TT (CC=major allele).
[b] AA vs AC vs CC (CC=major allele).

DISCUSSION AND CLINICAL IMPLICATIONS

COPEPTIN, DIABETES AND ABDOMINAL OBESITY

One of the key findings in this thesis was that copeptin predicted development of DM independently of renal function and a broad range of DM risk factors at baseline including FBG and insulin. Furthermore, we were able to reproduce the association between copeptin and the register-based DM end point using a reinvestigation-based end point (including measurement of OGTT) in a subset of MDC-CC (Study IV), which strongly supported the validity of our findings by showing that whichever method used to retrieve incident cases of DM, copeptin was an independent predictor of DM.

Another key finding of this thesis was that copeptin at baseline predicted development of abdominal obesity after 15.8 years of follow-up, and this finding was independent of renal function and baseline MetS components including waist circumference (Study IV).

Copeptin is a better predictor of diabetes in individuals without impaired fasting glucose

In Study II, we found the association between baseline copeptin and incident DM to be stronger in those individuals free of IFG at baseline, a finding that excluded the possibility that the association between copeptin and increased risk of DM was driven by individuals already having elevated FBG at baseline. This is important since AVP secretion is osmotically driven, and even though it is unlikely that elevated glucose levels would increase AVP release in a DM or pre-DM state (Figure 3), there may be other solutes elevated in DM that stimulate the osmotic AVP secretion, for example amino acids [137].

As expected, FBG was the strongest risk factor for new-onset DM. Each one mmol/L increase in FBG at baseline increased the risk of future DM with an OR of 11.4 (95% CI 7.4-17.5) in the fully adjusted model. Still, after accounting for FBG and all other available DM risk factors, individuals in the top quartile of copeptin had a two- to three-fold increased risk of developing DM compared with those in the lowest quartile of copeptin. In subjects without IFG, there was a significant improvement of the area under the ROC curve when copeptin was added to classic DM risk factors, consistent with an improvement in discrimination. The improvement in the area under the ROC curve was smaller when the entire

sample, including those with IFG, was considered. This finding may result from FBG being a more potent predictor of DM at FBG levels near the diagnostic limit of DM, whereas markers that are not part of the diagnostic criteria for DM, such as copeptin, may better signal DM susceptibility earlier in the disease state. Thus, our findings suggest that copeptin could provide valuable information for the prediction of DM, and it is possible that copeptin is especially useful as screening tool for finding "hidden high risk individuals" among individuals with normal FBG and thus on the average have low risk of developing DM. In the future, copeptin may be helpful in the evaluation of which individuals would, perhaps despite a normal FBG, benefit from undergoing an OGTT. Furthermore, copeptin may work as a motivation tool to incite individuals with unhealthy life style to make life style changes earlier.

Possible mechanisms behind vasopressin induced diabetes and abdominal obesity

We do not know whether AVP induces abdominal obesity and DM independently of each other or whether development of the two closely related traits is dependent upon each other. The association between baseline copeptin and incident DM was independent of incident abdominal obesity and vice versa, suggesting that AVP triggers two different pathways leading to DM and abdominal obesity independently. However, as we do not have repeated measurements of waist and glucose levels during follow-up, we cannot exclude the possibility that elevation of AVP induces increasing abdominal fat depositions, which in turn leads to DM development.

Metabolic effects of AVP are to be expected, but the contribution of AVP to glucose and lipid metabolism seems rather complex, and the exact mechanisms that underlie development of DM and abdominal obesity are not known. AVP mediates gluconeogenesis and glycogenolysis through the V1aR in the liver [48, 49] and stimulates the secretion of either glucagon or insulin, depending on the actual level of glycaemia, through the V1bR in pancreatic islets [50]. Furthermore, AVP exerts an anti-lipolytic action in rat, possibly through haemodynamic effects [85]. In addition, upon stressful stimuli, AVP binding to V1bR in the anterior pituitary gland mediates ACTH release and elevate glucocorticoid levels in plasma. Indeed, mice lacking the V1bR show lower levels of corticosterone in plasma [73]. The AVP-induced ACTH release has been reported to be resistant to glucocorticoid feedback in contrast to the CRH-induced ACTH release [83], suggesting that excessive AVP release, induced by stress or by a compensatory mechanism, overstimulates the HPA axis. Elevated

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glucocorticoid levels affects glucose and fat metabolism and leads to development of overweight, obesity and insulin resistance [138] and a mild Cushing-like phenotype. Thus, as different types of psychosocial stress are suggested to be associated with DM [139], our findings may point at the AVP system as a possible link between stress and metabolic dysfunction.

Moreover, mice with selective deletion of V1aR display elevated levels of AVP, high glucose levels, glucose intolerance and insulin resistance, predisposition for DM and obesity on the basis of a high fat intake, as well as low TG levels and enhanced lipid metabolism [69, 84]. On the contrary, mice lacking V1bR have the opposite phenotype of lower FBG and improved insulin sensitivity [140]. It can be speculated that a compensatory rise in AVP, perhaps as a result of AVP resistance for example at the level of V1aR or V2R, contribute to DM and/or abdominal obesity through stimulation of V1bR. In fact, selective V2R antagonism has in previous studies been shown to elevate AVP levels [141], and the commercially available V2R antagonist Tolvaptan have been associated with a 5% incidence of hyperglycemic events during 30 days of treatment in patients with hyponatremia compared with 1% in control subjects, although the numbers were small (12 of 223 versus 2 of 220) [142].

The vasopressin system as a potential treatment target

Copeptin was elevated long before the development of DM and abdominal obesity, and remained significantly associated independently of a broad range of potential confounders, suggesting a primary role of the AVP system in the pathophysiology of those endpoints. The AVP system is pharmacologically modifiable [142, 143]. Should future studies support causality between elevated AVP/copeptin levels and metabolic diseases, the AVP system, and perhaps the V1bR in particular, is an attractive target for interventions.

Moreover, copeptin is implicated in CVD [97-104], and it is possible that the incompletely understood interactions between DM and cardiovascular morbidity and mortality may partly be explained by a dysregulated AVP system.

Genetic variance in vasopressin receptors and the link to glucose and fat metabolism

Given the results of the AVP knockout mice models, we wanted to know if there were any links between genetic variance in the AVP receptors and altered glucose and fat metabolism in humans. In Study I, we found that the rs1042615 T-allele of the human AVPR1A gene in the MDC-CC population was associated with features resembling the phenotype of the male mice with V1aR deletion, including elevated FPG levels and low TG levels, as well as increased DM prevalence in subjects with a high-fat intake or overweight, and that these findings were driven by the male subset of the population. Interestingly, the rs1042615 T-allele was also recently found to be associated with higher BMI among Japanese men [86]. The cause of the relatively higher FPG and lower fasting TG concentrations in human carriers of the rs1042615 T-allele may have an etiology similar to that of V1aR knockout mice, i.e. preferential metabolism of fat instead of glucose, possibly as a consequence of reduced expression of the V1aR.

In Study V, the key finding was that the major allele of rs35810727 of the human AVPR1B gene was associated with elevated BMI both in MDC-CC and in the MDC replication cohort. Furthermore, in the complete MDC cohort the major allele of rs35810727 was associated with DM. Our results may be linked to an excessive activity of the V1bR mediated ACTH release from the anterior pituitary gland. One could speculate that carriers of the major allele of the AVPR1B tag SNP rs35810727 have enhanced AVPR1B signalling, either due to gain of V1bR receptor function, or enhanced gene expression. This would be expected to result in excessive V1bR mediated ACTH release and a Cushing-like phenotype.

Thus, we have identified genetic variants in both the AVPR1A and the AVPR1B genes that are involved in body weight and glucose regulation. Our results once again suggest a causal relationship between weight gain, DM and disturbance of the AVP system.

Analyses of genetical predisposition to altered copeptin levels

The genetically driven alterations of the metabolic phenotype among T-allele carriers of the AVPR1A rs1042615 and among carriers of the major allele of AVPR1B rs35810727, as well as our findings that copeptin is associated with DM and abdominal obesity, may partly be explained by genetically driven compensatory changes of AVP/copeptin levels due to altered expression or function of AVP receptors. Indeed, the V1aR knockout mice express elevated AVP levels [84], suggesting that V1aR impairment is followed by a compensatory rise in AVP levels and at the

same time development of metabolic abnormalities. We did not find any elevated copeptin levels in the AVPR1A rs1042615 T-allele carriers that would support this theory. On the contrary, we found significantly lower levels of copeptin among male T-allele carriers (unpublished data, Table 8). Neither did we find any association between the AVPR1B rs35810727 and altered copeptin levels (Table 8).

We conclude that any genetically driven alteration of AVP/copeptin levels, and its potential clinical relevance, must be re-evaluated and verified in future studies.

COPEPTIN AND THE METABOLIC SYNDROME

The causes of the clustering of cardiovascular risk factors within the MetS are still unknown, and in order to identify new treatment targets it is necessary to identify underlying pathophysiological factors. Recently, a cross-sectional association was found between plasma copeptin and MetS and components of the MetS in a population with familial hypertension and a relatively high prevalence of MetS (~50%) [144]. In Study III, we extended these findings as well as our own previous findings that copeptin was cross-sectionally associated with hyperinsulinemia and DM, by showing that high copeptin in a population-based sample was associated both with the clustering of the MetS components and also showed independent relationships with core components of the syndrome, *i.e.* hypertension and abdominal obesity, as well as with elevated CRP, after adjustment for each other as well as for DM and insulin resistance. These results suggested the AVP system as a unifying etiological link behind MetS, even though the cross-sectional design made it impossible to draw conclusions about causality. Thus, we conducted a prospective study relating plasma copeptin level to future development of MetS and its core components (Study IV), and found independent associations between baseline copeptin and development of abdominal obesity and DM at reinvestigation. Interestingly, despite the prospective relationship between copeptin and those two core components of MetS and our previously observed cross-sectional association between copeptin and MetS, we did not find copeptin to be an independent predictor of the cluster of MetS (Study IV). We thus believe our cross-sectional finding, relating copeptin with the cluster of MetS, was driven by the association between copeptin and both DM and abdominal obesity, respectively.

In Study III, the environmental factors low physical activity, high fat intake, and low socioeconomic status, which are all implied in MetS, were found to be associated with elevated copeptin levels. However, adjustment for these environmental factors did not affect the cross-sectional associations between copeptin and MetS or components of the MetS. This argues against a major role of those environmental factors in explaining the copeptin-MetS relationship. However, we could not completely exclude such a role due to the cross-sectional design of Study III. Thus, we conducted prospective analyses in the MDC reinvestigation cohort relating plasma copeptin level to future development of the two metabolic syndrome components shown to be longitudinally associated with copeptin level, i.e. DM and abdominal obesity. After adjustment for age, sex, follow-up time, low physical activity, high fat intake, and low socioeconomic status among subjects without DM at baseline (n=1928), the increase per

copeptin quartile (expressed as OR and 95% CI) of DM at reinvestigation was 1.19 (1.06-1.34), P trend=0.003. When analyzing men and women separately, the association remained borderline significant in men and significant in women [1.16 (0.97-1.38), P trend=0.10 and 1.23 (1.05-1.44), P trend=0.01, respectively]. Among subjects without abdominal obesity and without known DM at baseline (n=1779), the increase per copeptin quartile (expressed as OR and 95% CI) of abdominal obesity at reinvestigation was 1.14 (1.03-1.25) P trend=0.009. The analysis remained significant and borderline significant when conducted in men and women separately [1.19 (1.01-1.41), P trend=0.04 for men and 1.12 (0.99-1.26), P trend=0.06 for women] (unpublished data).

COPEPTIN AND MICROALBUMINURIA

Apart from constituting the core of the diagnosis of diabetic nephropathy, microalbuminuria is considered as an early sign of renal disorder and organ damage of the cardiovascular system, and it is an independent predictor of CVD [145, 146].

Our finding in Study IV, that copeptin level at baseline is associated with microalbuminuria after long-term follow-up independently of baseline MetS variables and CRP supports previous cross-sectional findings in humans [90, 147] and suggests a role of the AVP system in the development of microalbuminuria.

The association between copeptin and microalbuminura could be speculated to be explained by AVP-mediated changes of glucose metabolism or BP during follow-up. However, the association was present whether or not subjects with DM at baseline were included, and it remained significant after adjustment for all MetS factors, cystatin C and CRP at baseline, as well as for both incident DM and hypertension. This suggests that development of microalbuminuria may be at least partly directly mediated by AVP and not dependent on other cardiometabolic risk factors related to copeptin such as DM and hypertension. Regarding the pathway whereby AVP may increase albumin excretion, convincing data from humans and animals suggest that AVP contribute to albumin excretion as a consequence of its antidiuretic effect mediated by the V2R [147-149]. In addition, AVP suppression lowered proteinuria and improved renal function in rats with subtotal nephrectomy [150, 151]. Conversely, chronic infusion of dDAVP, a V2R agonist, exaggerated proteinuria in a rat model of renal failure [152]. These data add to the picture of a harmful role of V2R mediated AVP effects in the kidney.

Thus, in contrast to the relationship between AVP, DM and abdominal obesity, which is most likely dependent on V1aR and/or V1bR effects, experimental evidence point at the V2R as a link between AVP and microalbuminuria.

SUMMARY AND CONCLUSIONS

The key findings of this thesis are that elevated copeptin in plasma at baseline, a marker of AVP release, independently predict incident DM, abdominal obesity and microalbuminuria at reinvestigation (15.8 years of follow-up) (Study IV). The findings concerning DM and abdominal obesity are also supported by cross-sectional findings in Study II and III. Furthermore, the finding that copeptin predict DM is originally discovered and validated in Study II where the DM endpoint is based on register data.

The key findings in our genetic studies are that 1) the AVPR1A rs1042615 T-allele is associated with features resembling the phenotype of mice lacking the V1aR, including elevated glucose and low TG levels and an increased prevalence of DM in subjects with a high fat intake or who are overweight and 2) the major allele of AVPR1B rs35810727 is associated with elevated BMI, and our results also indicate a link between AVPR1B variance and DM development.

On the basis of the analyses in this large population-based cohort we conclude that

1) Elevated copeptin is an independent predictor of DM and abdominal obesity, and is associated with microalbuminuria after long term follow-up. These findings implicate that the pharmacologically modifiable AVP system may be a new treatment target for these conditions, which are all important risk factors for CVD.

2) Copeptin predicts DM development long before development of IFG. In selected groups of individuals, copeptin may be a useful early marker to evaluate future risk of DM or to evaluate the need of further screening for DM.

3) Genetic variance in the AVP system is associated with body weight regulation and DM development. These data suggest a causal relationship between disturbance of the AVP system and development of potentially modifiable cardiovascular risk factors.

4) Our findings highlight the possibility that pharmacotherapy which selectively antagonize the AVP system may have metabolic side effects.

5) Increased intake of water may be beneficial as it would decrease levels of plasma AVP/copeptin.

FUTURE RESEARCH

This thesis has established a link between copeptin and cardiovascular risk factors. We are now planning to investigate the associations between copeptin and hard endpoints, for example CVD, total mortality and major adverse cardiac events in the MDC-CC population.

Our findings are in line with findings by others and have partly been replicated [86, 90, 144, 153]. Furthermore, our results rely on data collected from a large prospective cohort study. This is why it is reasonable to assume that alterations in the AVP system, measured as elevated copeptin levels, are causally associated with development of DM and abdominal obesity and not only a matter of co-variation. We believe there are several ways to eventually find out if the associations between copeptin and the cardiometabolic risk factors are causal:

1) We are conducting an experimental intervention study to find out if life style factors, for example extensive water drinking, may alter copeptin levels in parallel with a decreased cardiometabolic risk. Previous data have demonstrated that copeptin level in plasma decreases during hypotonic saline infusion in combination with desmopressin administration [154], and in a pilot study drinking 1 L of water caused a fall in plasma copeptin within one hour [88].

2) In the long run, we would like to test pharmacological manipulation studies in which we use different combinations of AVP receptor stimulation and inhibition. By concomitant measuring of different cardiometabolic risk factors we aim to define which copeptin/AVP effects that are mediated through the different AVP receptors.

3) We are planning a mendelian randomization study to find out whether individuals that are genetically predisposed to elevated copeptin levels are also prone to a disadvantageous cardiometabolic risk factor profile. If we find copeptin-associated loci that are also robustly associated with cardiometabolic risk factors, we may assume causality between these traits, as the individuals that are genetically predisposed to elevated copeptin levels has been so since birth, which excludes the possibility of confounding from environmental factors.

SUMMARY IN SWEDISH

Svensk sammanfattning

Kroniska sjukdomar orsakar ungefär 35 miljoner dödsfall runt om i världen årligen, vilket utgör ungefär 60 % av det totala dödstalet. De största grupperna av kronisk sjukdom är i tur och ordning hjärt-kärlsjukdom, cancer, kroniska lungsjukdomar och diabetes. Övervikt, fetma och diabetes är ett växande problem över hela världen, och är samtidigt viktiga anledningar till den ökande förekomsten av hjärt-kärldöd.

Vasopressin är mest känt som ett hormon som reglerar kroppens salt-vätske-balans via effekter i njuren. Idén att undersöka vasopressin-hormonets kopplingar till metabola sjukdomar såsom diabetes och övervikt kom från japanska experiment på möss där man funnit att vasopressin påverkar kroppens fett- och sockeromsättning. Det har tidigare varit svårt att studera vasopressin i stor utsträckning eftersom tillförlitliga mätmetoder av proteinet har saknats och halveringstiden i blodet är mycket kort. En ny metod är nyligen utvecklad för att enkelt uppskatta blodets innehåll av vasopressin, och går ut på att istället mäta ett protein som bildas samtidigt som vasopressin, s.k. copeptin. Nivåerna av copeptin i blodet speglar kroppens vasopressin-frisättning.

Malmö-kost-cancer-studien utgörs av 30447 slumpvis utvalda malmöbor som mellan 1991 och 1996 lämnade blodprover och DNA-prov, genomgick mätning av vikt, längd och blodtryck och besvarade ett utförligt frågeformulär som bland annat behandlade medicinering, motionsvanor och kostvanor. Av dessa utgör 6103 individer den så kallade hjärt-kärl-kohorten. Dessa individer genomgick förutom den basala undersökningen och frågeformuläret även en ultraljudsundersökning av de stora halskärlen och lämnade fasteblodprover. I upptinade blodprover från stora delar av denna hjärt-kärl-kohort uppmättes copeptin. Delar av de 6103 individerna har ca 16 år senare återundersökts med bland annat vikt, längd och fastande blodprover.

Med hjälp av nationella och regionala diabetesregister, samt med hjälp av återundersökningen, har vi kunnat studera vilka individer som har utvecklat diabetes och övervikt, och relatera detta till deras copeptin-nivåer 16 år tidigare. Vi har även undersökt hur individernas sjukdomsbild påverkas av genetiska varianter i två gener som kodar för vasopressin-receptorer, det vill säga de proteiner som vidarebefordrar signaler från vasopressin.

Vi undersökte i studie I variation i generna som kodar för den vasopressin-receptor som benämns 1a. Vi fann att en specifik genvariant ledde till att de individer som bar denna variant hade förhållandevis högre sockernivåer i

blodet och, om de samtidigt åt mycket fett i relation till övrigt matintag, så hade de även ökad risk för diabetes. Dessa egenskaper var mycket lika de egenskaper som i japanska studier uppvisades av specialdesignade möss som sedan födseln saknade vasopressin-receptor 1a.

I studie II visade vi att nivån i blodet av vasopressin, mätt som copeptin, förutspår framtida diabetes. Vi fann att copeptin förutspår framtida diabetes långt innan blodsockret börjar stiga. Höga copeptin-nivåer var förenat med en mellan 2 och 3.5 gånger så hög risk att insjukna i diabetes jämfört med låga copeptin-nivåer. Copeptin var ett bättre mått på diabetesrisk hos de personer som vid studiestarten hade ett helt normalt fasteblodsocker än hos de som hade gränsvärdesförhöjt fasteblodsocker. Sambandet mellan copeptin och framtida diabetes var helt oberoende av kända riskfaktorer för diabetes såsom blodsockernivå, insulinresistens, övervikt, ärftlighet, inflammation, blodfetter, högt blodtryck, ålder och rökning.

I studie III och IV fann vi att högt copeptin var kopplat inte bara till diabetes, utan även till andra sjukdomar som ingår i det syndrom som brukar kallas för metabola syndromet, nämligen högt blodtryck, fetma, bukfetma och generell inflammation. Med hjälp av data från återundersökningen fann vi också att högt copeptin förutspådde framtida bukfetma och påverkade njurfunktionen.

Studie V var ytterligare en genetisk studie. Denna gång undersökte vi genetisk variation i den vasopressin-receptor som benämns 1b. Vi fann att de som ärvde en viss genvariant i denna receptor hade ökad risk för att utveckla övervikt.

Vasopressin produceras i hjärnan och ökar exempelvis vid olika former av stress. En möjlig förklaring till våra fynd är att vasopressin aktiverar och förstärker effekten av andra stresshormoner som kan bidra till både diabetes och viktuppgång. Copeptinets förmåga att förutspå framtida diabetes var bäst hos människor med fullständigt normala blodsockernivåer, och vi tror att mätning av copeptin i blodet i framtiden kan användas för att tidigt hitta friska människor med hög risk för diabetes. Vi kommer därför nu att studera om man kan förebygga övervikt, diabetes och hjärt-kärlsjukdomar genom att på ett tidigt stadium dämpa vasopressin-effekterna i kroppen. Intressant nog finns det redan läkemedel som blockerar vasopressinets effekter och används vid behandling av salt-vätske-rubbningar och hjärtsvikt. Dessutom har ett flertal livsstilsfaktorer såsom exempelvis motion och vattenintag visats påverka kroppens vasopressin-frisättning.

Sammanfattningsvis så har vi visat att det finns många kopplingar mellan det salt-vätske-reglerande hormonet vasopressin, dess receptorer, och dålig socker- och viktkontroll. Vi har funnit att nivån av copeptin, en

vasopressin-markör, förutspår både framtida diabetes och viktuppgång. Övervikt ökar risken för både diabetes och hjärt-kärsjukdomar. Diabetes ökar kraftigt risken för hjärt-kärlsjukdomar, men sänkning av blodsockret har inte visats minska risken för hjärt-kärldöd. Eftersom höga copeptin-nivåer tidigare har visat sig vara kopplat till prognos vid hjärtinfarkt och hjärtsvikt tror vi att vasopressin är en viktig och potentiellt behandlingsbar länk mellan diabetes och dess viktigaste komplikation, hjärt-kärlsjukdom.

ACKNOWLEDGEMENTS

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Relation between human vasopressin 1a gene variance, fat intake, and diabetes[1-3]

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ABSTRACT

Background: Male arginine vasopressin 1a receptor knockout mice (V1aR$^{-/-}$) display a phenotype of low triglycerides and high glucose concentrations and high-fat-diet–induced obesity and diabetes.

Objective: We investigated whether genetic variation of the human arginine vasopressin 1A (*AVPR1A*) gene is associated with phenotypic features resembling those of the V1aR$^{-/-}$ mouse.

Design: In a population-based cross-sectional study in southern Sweden, middle-aged individuals ($n = 6055$) were examined in 1991–1994. Associations between 4 *AVPR1A* tag single nucleotide polymorphisms (rs1042615, rs10784339, rs7308855, and rs10747983) and diabetes status, glucose and triglyceride concentrations, and BMI were analyzed. Furthermore, rs1042615 was related to diabetes status, glucose, and triglycerides within sex-specific quartiles of dietary fat intake ($Q1_{Fat}$-$Q4_{Fat}$) and BMI ($Q1_{BMI}$-$Q4_{BMI}$).

Results: Subjects carrying the *T* allele of rs1042615 had lower concentrations of triglycerides than did *CC* carriers (1.36 ± 0.77 compared with 1.42 ± 0.89 mmol/L; $P = 0.014$), especially in nondiabetic subjects ($P = 0.001$). Carriers of the rs1042615 *T* allele had higher fasting blood glucose (5.20 ± 1.44 mmol/L compared with 5.12 ± 1.22 mmol/L; $P = 0.036$) and a tendency toward an increased prevalence of diabetes (odds ratio: 1.22; 95% CI: 0.99, 1.51; $P = 0.067$) compared with *CC* carriers. The less common rs10784339, rs7308855, and rs10747983 were not consistently associated with metabolic variables. Among men, the rs1042615 *T* allele was associated with diabetes exclusively within $Q4_{Fat}$ (odds ratio: 2.22; 95% CI: 1.05, 4.71; $P = 0.04$) and $Q4_{BMI}$ (odds ratio: 1.81; 95% CI: 1.11, 2.93; $P = 0.02$).

Conclusion: The rs1042615 *T* allele is associated with features resembling the phenotype of the V1aR$^{-/-}$ mouse, including uncoupling of the usual direct relation between glucose and triglycerides and an increased prevalence of diabetes in subjects with a high fat intake or who are overweight. *Am J Clin Nutr* 2009;89:1–7.

INTRODUCTION

Type 2 diabetes mellitus and its closely related conditions of obesity and hypertriglyceridemia are all highly heritable (1, 2). A combination of environmental factors and many different gene variants are likely to interact in determining the level of glycemia, body mass index (BMI; in kg/m^2), and triglyceridemia.

The neurohypophyseal peptide arginine vasopressin (AVP) is involved in the inhibition of diuresis, modulation of ACTH release, stimulation of liver glycogenolysis, facilitation of thrombosis, and contraction of smooth muscle. These effects are mediated through 3 different vasopressin receptors: V1aR, V2R, and V1bR (also called V3) (3, 4). In patients with type 2 diabetes, the plasma AVP concentration has been reported to be elevated (5), and AVP infusion in humans leads to elevated circulating glucose concentrations (6). Thus, apart from an osmotically induced increase in AVP secretion due to hyperglycemia (7), it can be hypothesized that AVP or altered AVP sensitivity at the receptor or postreceptor level may be involved in regulating glucose homeostasis in humans.

Recent data show significant changes in the lipid and glucose metabolism of male mice lacking one of the vasopressin receptors—V1aR (V1aR$^{-/-}$). On a normal Chow diet, these knockout mice had slightly higher fasting glucose concentrations and glucose intolerance than did wild-type (WT) mice (8). Interestingly, in contrast with what would be expected from the direct relation between circulating concentrations of glucose and triglycerides in human epidemiologic studies, V1aR$^{-/-}$ mice had lower concentrations of circulating plasma triglycerides than did WT mice (9), which suggested that the loss of V1aR function can uncouple the usual positive correlation between these 2 traits. The V1aR$^{-/-}$ mice weighed less than the WT mice after 16 wk of a normal Chow diet, but after being fed a high-fat diet the V1aR$^{-/-}$ mice were more prone to develop overt obesity and diabetes (8).

Given the phenotype of the V1aR$^{-/-}$ mice, we hypothesized that the common genetic variation of the human homolog to the *V1aR* gene, ie, the arginine vasopressin 1A (*AVPR1A*) gene, in a population-based sample would be associated with a similar phenotype of elevated glucose concentrations accompanied by reduced concentrations of triglycerides, although less pro-

nounced than the complete knockout effect seen in V1aR$^{-/-}$. Furthermore, the finding that a high-fat diet in the V1aR$^{-/-}$ mice resulted in exacerbation of overt obesity and diabetes prompted us to test the hypothesis that any association between genetic variation in the *AVPR1A* gene and impaired glucose homeostasis would be enhanced in subsets of our population with the highest intake of dietary fat.

SUBJECTS AND METHODS

Subjects

The Malmö Diet and Cancer (MDC) Study is a population-based prospective cohort consisting of 28,449 persons surveyed in 1991–1996 (10). From this cohort, 6103 persons were randomly selected and referred to as the MDC cardiovascular arm (MDC-CVA; $n = 6103$, examined 1991–1994, DNA available on $n = 6055$). Data from MDC-CVA are used to investigate risk factors for cardiovascular disease. Fasting blood samples for analyses of glucose and blood lipids were obtained from the majority ($n = 5506$).

Glucose and triglyceride analyses were carried out at the Department of Clinical Chemistry, Malmö University Hospital, which is attached to a recurrent standardization system. Glucose was measured in overnight fasting blood samples in whole blood glucose by a hexokinase-glucose-6-phosphate dehydrogenase method. Diabetes mellitus was defined by self report of a physician diagnosis or fasting blood glucose concentration ≥ 6.1 mmol/L or use of antidiabetic medication. Overweight was defined as a BMI ≥ 25 and obesity as a BMI ≥ 30. Clinical characteristics of study subjects are shown in **Table 1**. The study protocols were approved by the ethics committee of Lund University, and all participants provided written informed consent.

Relative dietary fat intake

Dietary data were collected in 5745 individuals through a modified diet history, combining *1*) a menu book for recording cooked lunch and dinner meals, drugs, natural remedies, nutrient supplements, and cold beverages, including alcohol, consumed during 7 consecutive days; *2*) a 168-item questionnaire for assessment of meal pattern, consumption frequencies, and portion sizes of regularly eaten foods (the reference period was the preceding year); and *3*) a 45-min complementary interview focusing on cooking practices and portion sizes in the menu book (11). Photographic aids were used to help with the assessment of portion sizes. The consistency of the information provided was carefully checked so that the questionnaire and menu-book did not overlap. The mean daily intake of foods was calculated on the basis of frequency and portion size estimates from the questionnaire and menu book. The food intake information was converted into nutrient intake data by using the MDC nutrient database, from which most of the nutrient information comes from PC-KOST2-93 from the National Food Administration in Uppsala, Sweden.

The relative validity of the MDC method was evaluated in 1984–1985 in a sample of Malmö residents (105 women and 101 men) aged 50–69 y using 18 d of weighed records, 3 d every second month during a year, as the reference method. The Pearson correlation coefficients for total fat, adjusted for total energy, between the reference method and the MDC method were 0.69 in women and 0.64 in men (12). These values are generally higher than those found with the use of comparable dietary methods in other studies, which were performed in other populations (13).

Because dietary patterns and self-reported dietary intakes tend to differ according to sex (14, 15), the subgroup analyses of genotype-phenotype associations in different quartiles of fat intake were analyzed in male and female subjects separately. After logarithmic transformation, we regressed total fat intake on total energy intake in males and females separately. The residuals were saved and used to rank individuals. The study population was divided into quartiles (Q1$_{Fat}$-Q4$_{Fat}$), with Q1$_{Fat}$ representing those with the lowest and Q4$_{Fat}$ those with the highest relative intake of dietary fat. Using strata of residuals of fat intake in relation to total energy intake (Q1$_{Fat}$-Q4$_{Fat}$), instead of total fat intake, we reduced confounding from dietary over- and underreporting. Furthermore, as a diagnosis of diabetes commonly leads to a change in dietary pattern, subjects with known diabetes (ie, reporting a history of diabetes or being under treatment with antidiabetic agents) were excluded in the fat intake stratified analyses of genotype compared with diabetes and fasting blood glucose concentration ($n = 5188$). Thus, in these analyses, all diabetes cases were new onset diabetes cases defined solely by having fasting blood glucose ≥ 6.1 mmol/L on baseline screening.

Leisure-time physical activity was assessed on the basis of a list of activities (18 items) adapted from the Minnesota Leisure Time Physical Activity instrument (16). A score was obtained by multiplying the reported amount of minutes per week spent on a specific activity by an activity-specific factor. Based on this score, the population was ranked into quartiles.

Genotyping

DNA was extracted from frozen granulocyte or buffy coat samples collected from MDC-CVA with the use of QIAamp-96 spin blood kits (QIAGEN, Stockholm, Sweden) at the DNA extraction facility supported by SWEGENE. To analyze the AVPR1A polymorphism and capture the maximum of the genetic variance of the *AVPR1A* gene, data from HapMap were used (www.hapmap.org) to select 4 tag single nucleotide polymorphisms (SNPs): rs1042615, rs10747983, rs10784339, and rs7308855. Primers and probes were custom synthesized by Applied Biosystems (Foster City, CA) according to standard recommendations for the AB Prism 7900HT analysis system, and genotyped with polymerase chain reaction–based methods (17).

TABLE 1
Characteristics of the population description ($n = 6055$)

Characteristic	Value
Age (y)	57.5 ± 5.9[1]
Sex (% men)	42.2
BMI (kg/m^2)	25.9 ± 4.0
Obesity (%)[2]	13.5
Diabetes (%)[3]	8.8
Triglycerides (mmol/L)	1.38 ± 0.80
Glucose (mmol/L)	5.18 ± 1.38

[1] Mean ± SD (all such values).
[2] Defined as a BMI ≥ 30 kg/m^2.
[3] Defined as self-report of a physician diagnosis or a fasting blood glucose concentration ≥ 6.1. mmol/L or use of antidiabetic medications.

Statistics

SPSS statistical software (version 14.0; SPSS Inc, Chicago, IL) was used for all calculations. Data are expressed as means ± SDs. Because fasting concentrations of blood glucose and plasma triglycerides tended to be skewed to the right, all statistical analyses were performed after natural logarithmic transformation of the 2 traits. Significances of differences in continuous variables were tested with a *t* test or analysis of variance (ANOVA). Multivariate linear regression was used to test whether associations between genetic variants and continuous variables were independent of potential confounders. Significance of frequency differences in dichotomous variables was tested with a chi-square test. Multivariate logistic regression analysis was used to estimate odds ratios (ORs) and 95% CIs for dependent dichotomous variables in relation to genetic variance in crude and adjusted models. To test whether genetic associations differed according to fat intake and BMI, we stratified our population into quartiles of fat intake ($Q1_{Fat}$, $Q2_{Fat}$, $Q3_{Fat}$, and $Q4_{Fat}$) and BMI ($Q1_{BMI}$, $Q2_{BMI}$, $Q3_{BMI}$, and $Q4_{BMI}$). A 2-sided *P* value <0.05 was considered statistically significant.

RESULTS

The genotyping success rate was 96.8% (rs1042615), 96.3% (rs10747983), 97.1% (rs10784339), and 97.0% (rs7308855). Genotype frequencies did not deviate from Hardy-Weinberg equilibrium (*P* > 0.60 for all SNPs).

Genetic *AVPR1A* variance vs triglycerides

Carriers of at least one rs1042615 *T* allele had significantly lower concentrations of triglycerides (**Table 2**). After adjustment for age, sex, physical activity, and BMI, the association was still significant (*P* = 0.025). Further inclusion of glucose in the model strengthened the association even more (*P* = 0.001).

Because triglyceride concentrations are commonly elevated in diabetes as a result of hyperglycemia per se, we also tested the association between the rs1042615 *T* allele and triglycerides among nondiabetic subjects. We found that carriers of 1 or 2 copies of the rs1042615 *T* allele had highly significantly lower triglyceride concentrations than did carriers of the *CC* genotype (1.29 ± 0.68 mmol/L compared with 1.37 ± 0.84 mmol/L; *P* = 0.001).

None of the other *AVPR1A* tag SNPs (rs10747983, rs10784339, and rs7308855) were significantly associated with triglyceride concentrations (Table 2).

Genetic *AVPR1A* variance vs glucose homeostasis

Individuals carrying at least one rs1042615 *T* allele had significantly higher fasting blood glucose concentrations than did carriers of the *CC* genotype (Table 2). The association between the *T* allele and elevated fasting blood glucose concentrations remained significant after adjustment for age, sex, physical activity, and BMI (*P* = 0.036) and was strengthened after additional inclusion of triglycerides in the model (*P* = 0.005). The

TABLE 2
Genotype V1aR variation in relation to metabolic phenotype

Genotype	Triglycerides	Glucose	Diabetes	Obesity[1]	BMI[1]
	mmol/L	*mmol/L*	*%*	*%*	*kg/m^2*
rs1042615 (*CC* = 30.4%, *CT* = 49.2%, *TT* = 20.4%), *n* = 5506					
CC	1.42 ± 0.89[2]	5.12 ± 1.22	7.7	12.5	25.9 ± 3.8
CT	1.36 ± 0.79	5.21 ± 1.45	9.7	13.2	25.7 ± 4.0
TT	1.36 ± 0.71	5.19 ± 1.44	8.1	15.1	26.0 ± 4.1
P value[3]	0.037	0.11	0.052	0.12	0.10
P value[4]	0.014	0.036	0.067	0.19	0.74
rs10747983 (*AA* = 2.6%, *AG* = 26.8%, *GG* = 70.6%), *n* = 5506					
AA	1.45 ± 0.76	5.18 ± 1.35	12.3	13.1	25.7 ± 3.8
AG	1.40 ± 0.81	5.16 ± 1.41	8.1	12.3	25.8 ± 3.8
GG	1.37 ± 0.80	5.19 ± 1.39	9.1	13.9	25.9 ± 4.0
P value[3]	0.19	0.72	0.21	0.27	0.77
rs10784339 (*CC* = 2.6%, *CG* = 27.0%, *GG* = 70.4%), *n* = 5506					
CC	1.44 ± 0.76	5.13 ± 1.21	11.5	12.3	25.6 ± 3.8
CG	1.40 ± 0.81	5.16 ± 1.38	7.9	12.4	25.8 ± 3.8
GG	1.37 ± 0.80	5.19 ± 1.39	9.1	14.0	25.9 ± 4.0
P value[3]	0.27	0.59	0.22	0.26	0.61
rs7308855 (*CC* = 82.5%, *CT* = 16.8%, *TT* = 0.8%), *n* = 5506					
CC	1.37 ± 0.79	5.18 ± 1.37	9.1	13.5	25.9 ± 4.0
CT	1.42 ± 0.87	5.18 ± 1.51	8.1	13.2	25.9 ± 3.8
TT	1.39 ± 0.68	4.97 ± 0.68	8.6	11.1	25.8 ± 3.3
P value[3]	0.37	0.62	0.62	0.89	0.99

[1] *n* = 6055.

[2] Mean ± SD (all such values).

[3] ANOVA (for triglycerides, glucose, and BMI), chi-square test (for diabetes and obesity).

[4] *CC* vs *CT/TT* genotype: *t* test for triglycerides, glucose, and BMI and logistic regression for diabetes and obesity.

OR for diabetes in carriers of the *T* allele was 1.22 (95% CI: 0.99, 1.51; *P* = 0.067). *AVPR1A* tag SNPs rs10747983, rs10784339, and rs7308855 were neither significantly associated with fasting blood glucose concentration nor with diabetes status.

rs1042615 In relation to diabetes after stratification for fat intake

The finding that the phenotype of human carriers of the rs1042615 *T* allele (increased glucose and decreased triglyceride concentrations) resembled that of the V1aR$^{-/-}$ mouse encouraged us to test for further phenotypic similarities. Because male V1aR$^{-/-}$ mice were more prone than WT mice to develop overt diabetes and obesity when fed a high-fat diet, we tested the rs1042615 *T* allele for phenotypic association within sex-specific strata of fat intake (Q1$_{Fat}$-Q4$_{Fat}$). Because diabetes commonly leads to changes in dietary patterns, we excluded patients with a previous diagnosis of diabetes (79 men and 76 women).

In men within Q4$_{Fat}$, the rs1042615 *T* allele was significantly associated with diabetes, whereas there was no significant association in men belonging to Q1$_{Fat}$-Q3$_{Fat}$ (**Table 3**). An interaction test was conducted between the genetic variants and fat intake. The interaction term (fat intake × genotype) was entered into a multivariate logistic regression together with genotype and fat intake, with diabetes as the outcome variable. The interaction was significantly related to diabetes in men (*P* = 0.044). Among women, there was no association between the *T* allele and diabetes in any of the quartiles of fat intake (Table 3). After pooling men and women in Q4$_{Fat}$, we observed a borderline significantly higher prevalence of diabetes in *T* allele carriers than in carriers of the *CC* genotype (OR: 1.78; 95% CI: 0.99, 3.17; *P* = 0.052) that became significant after adjustment for sex, age, BMI, and physical activity (*P* = 0.013). In consequence, fasting blood glucose concentrations were slightly

TABLE 3
Differences in prevalence of diabetes between the *CT/TT* and *CC* genotypes by sex and quartile (Q) of fat intake in persons without known diabetes

Fat intake strata[1]	*CT/TT* genotype vs *CC* genotype[2]	*P* value[3]	*P* value[4]
Men (*n* = 2123)			
Q1$_{Fat}$ (*n* = 530)	0.85 (0.44–1.65)	0.64	0.54
Q2$_{Fat}$ (*n* = 535)	1.02 (0.47–2.25)	0.96	0.88
Q3$_{Fat}$ (*n* = 529)	1.34 (0.67–2.69)	0.41	0.46
Q4$_{Fat}$ (*n* = 529)	2.22 (1.05–4.71)	0.037	0.032
Women (*n* = 3065)			
Q1$_{Fat}$ (*n* = 764)	0.65 (0.30–1.41)	0.27	0.34
Q2$_{Fat}$ (*n* = 772)	1.18 (0.54–2.61)	0.68	0.66
Q3$_{Fat}$ (*n* = 773)	1.63 (0.73–3.62)	0.23	0.52
Q4$_{Fat}$ (*n* = 756)	1.42 (0.57–3.58)	0.45	0.22

[1] Q1$_{Fat}$-Q4$_{Fat}$ represents quartiles of relative dietary fat intake from the lowest to the highest intake. We regressed total fat intake on total energy intake in men and women separately, and the residuals were saved and used to rank individuals. Fat (g/d) in each quartile, expressed as median (interquartile range): Q1$_{Fat}$-Q4$_{Fat}$ for men—Q1$_{Fat}$ = 87 (71–105), Q2$_{Fat}$ = 106 (86–126), Q3$_{Fat}$ = 115 (97–145), and Q4$_{Fat}$ = 129 (105–160); Q1$_{Fat}$-Q4$_{Fat}$ for women—Q1$_{Fat}$ = 65 (54–80), Q2$_{Fat}$ = 78 (63–95), Q3$_{Fat}$ = 85 (72–102), and Q4$_{Fat}$ = 97 (79–118).
[2] All values are odds ratios (95% CIs).
[3] Logistic regression was used to analyze the data.
[4] *P* values in the logistic regression were adjusted for age, BMI, and physical activity.

TABLE 4
Differences in glucose and triglycerides between *CT/TT* and *CC* genotypes by sex and quartile (Q) of fat intake in persons without known diabetes

Fat intake strata[1]	*CT/TT* genotype	*CC* genotype	*P* value[2]	*P* value[3]
Glucose (mmol/L)				
Men (*n* = 2123)				
Q1$_{Fat}$ (*n* = 530)	5.15 ± 0.95[4]	5.21 ± 0.95	0.42	0.33
Q2$_{Fat}$ (*n* = 535)	5.23 ± 1.14	5.16 ± 1.34	0.33	0.29
Q3$_{Fat}$ (*n* = 529)	5.24 ± 0.86	5.21 ± 0.95	0.57	0.49
Q4$_{Fat}$ (*n* = 529)	5.27 ± 0.89	5.13 ± 0.57	0.080	0.061
Women (*n* = 3065)				
Q1$_{Fat}$ (*n* = 764)	4.87 ± 0.60	4.84 ± 0.64	0.39	0.22
Q2$_{Fat}$ (*n* = 772)	4.93 ± 0.67	4.86 ± 0.58	0.15	0.14
Q3$_{Fat}$ (*n* = 773)	4.91 ± 0.75	4.86 ± 0.56	0.40	0.73
Q4$_{Fat}$ (*n* = 756)	4.90 ± 0.70	4.94 ± 0.61	0.37	0.88
Triglycerides (mmol/L)				
Men (*n* = 1942)				
Q1$_{Fat}$ (*n* = 483)	1.44 ± 0.74	1.51 ± 0.70	0.21	0.17
Q2$_{Fat}$ (*n* = 500)	1.43 ± 0.81	1.52 ± 0.73	0.071	0.12
Q3$_{Fat}$ (*n* = 479)	1.41 ± 0.71	1.39 ± 0.66	0.95	0.70
Q4$_{Fat}$ (*n* = 480)	1.45 ± 0.82	1.69 ± 1.53	0.061	0.068
Women (*n* = 2934)				
Q1$_{Fat}$ (*n* = 733)	1.24 ± 0.62	1.24 ± 0.65	0.79	0.61
Q2$_{Fat}$ (*n* = 741)	1.18 ± 0.59	1.27 ± 0.74	0.15	0.17
Q3$_{Fat}$ (*n* = 733)	1.19 ± 0.57	1.19 ± 0.55	0.99	0.73
Q4$_{Fat}$ (*n* = 727)	1.16 ± 0.55	1.29 ± 0.69	0.013	0.064

[1] Q1$_{Fat}$-Q4$_{Fat}$ represents quartiles of relative dietary fat intake from the lowest to the highest intake. We regressed total fat intake on total energy intake in men and women separately, and the residuals were saved and used to rank individuals. Fat (g/d) in each quartile, expressed as median (interquartile range): Q1$_{Fat}$-Q4$_{Fat}$ for men—Q1$_{Fat}$ = 87 (71–105), Q2$_{Fat}$ = 106 (86–126), Q3$_{Fat}$ = 115 (97–145), and Q4$_{Fat}$ = 129 (105–160); Q1$_{Fat}$-Q4$_{Fat}$ for women—Q1$_{Fat}$ = 65 (54–80), Q2$_{Fat}$ = 78 (63–95), Q3$_{Fat}$ = 85 (72–102), and Q4$_{Fat}$ = 97 (79–118).
[2] A *t* test was used to analyze the data.
[3] *P* values in the logistic regression were adjusted for age, BMI, and physical activity.
[4] Mean ± SD (all such values).

higher in men carrying the *T* allele than in carriers of the *CC* genotype in Q4$_{Fat}$ (5.13 ± 0.57 mmol/L compared with 5.27 ± 0.89 mmol/L) with *P* = 0.08 in crude analyses and *P* = 0.061 in analyses adjusted for BMI and physical activity, whereas fasting glucose concentrations did not differ between carriers and noncarriers of the *T* allele among Q1$_{Fat}$-Q3$_{Fat}$ (**Table 4**).

rs1042615 In relation to diabetes after stratification for BMI

We thereafter tested whether the association of the rs1042615 *T* allele with diabetes is affected by stratification for BMI (Q1$_{BMI}$-Q4$_{BMI}$). BMI is unlikely to be subject to bias by a known diagnosis of diabetes, thus allowing us to include all diabetes patients. Similar to the association between the rs1042615 *T* allele and diabetes within Q4$_{Fat}$ in men, the association between the *T* allele and diabetes within Q4$_{BMI}$ was significant in men (**Table 5**). An interaction test, conducted in the same manner as the one for fat intake, showed that the BMI/genotype-interaction term was borderline significantly related to diabetes in men (*P* = 0.093). In concert with this finding, the *T* allele was in men associated with significantly higher fasting blood glucose within Q4$_{BMI}$ (**Table 6**), whereas there was no

TABLE 5
Differences in prevalence of diabetes between *CT/TT* and *CC* genotypes by sex and quartile (Q) of BMI

BMI strata[1]	*CT/TT* genotype vs *CC* genotype[2]	P value[3]	P value[4]
Men (*n* = 2280)			
Q1$_{BMI}$ (*n* = 559)	1.09 (0.45–2.61)	0.85	0.94
Q2$_{BMI}$ (*n* = 582)	0.84 (0.44–1.61)	0.60	0.57
Q3$_{BMI}$ (*n* = 584)	1.29 (0.74–2.26)	0.38	0.27
Q4$_{BMI}$ (*n* = 555)	1.81 (1.11–2.93)	0.017	0.013
Women (*n* = 3224)			
Q1$_{BMI}$ (*n* = 811)	0.81 (0.28–2.41)	0.71	0.53
Q2$_{BMI}$ (*n* = 807)	4.24 (0.98–18.36)	0.053	0.063
Q3$_{BMI}$ (*n* = 809)	0.96 (0.51–1.81)	0.90	0.95
Q4$_{BMI}$ (*n* = 797)	1.08 (0.71–1.65)	0.72	0.92

[1] Q1$_{BMI}$-Q4$_{BMI}$ represents quartiles of BMI (in kg/m^2) from the lowest to the highest BMI. Median (interquartile range): Q1$_{BMI}$-Q4$_{BMI}$ for men— Q1$_{BMI}$ = 22.6 (21.5–23.4), Q2$_{BMI}$ = 24.9 (24.4–25.5), Q3$_{BMI}$ = 27.0 (26.5–27.6), and Q4$_{BMI}$ = 30.1 (29.1–31.6); Q1$_{BMI}$-Q4$_{BMI}$ for women— Q1$_{BMI}$ = 21.1 (20.2–22.0), Q2$_{BMI}$ = 23.7 (23.2–24.2), Q3$_{BMI}$ = 26.1 (25.5–26.9), and Q4$_{BMI}$ = 30.5 (28.9–32.8).
[2] All values are odds ratios (95% CIs).
[3] Logistic regression was used to analyze the data.
[4] P values in the logistic regression were adjusted for age and physical activity.

TABLE 6
Differences in glucose and triglyceride concentrations between *CT/TT* and *CC* genotypes by sex and quartile (Q) of BMI

BMI strata[1]	*CT/TT* genotype	*CC* genotype	P value[2]	P value[3]
Glucose (mmol/L)				
Men (*n* = 2279)				
Q1$_{BMI}$ (*n* = 560)	5.16 ± 1.66[4]	5.09 ± 1.22	0.71	0.85
Q2$_{BMI}$ (*n* = 580)	5.13 ± 1.22	5.22 ± 1.24	0.30	0.34
Q3$_{BMI}$ (*n* = 583)	5.46 ± 1.28	5.32 ± 1.19	0.12	0.086
Q4$_{BMI}$ (*n* = 556)	6.04 ± 2.14	5.54 ± 1.47	0.003	0.006
Women (*n* = 3220)				
Q1$_{BMI}$ (*n* = 810)	4.78 ± 1.03	4.71 ± 0.64	0.32	0.46
Q2$_{BMI}$ (*n* = 806)	4.88 ± 1.16	4.76 ± 0.46	0.14	0.11
Q3$_{BMI}$ (*n* = 808)	5.02 ± 1.11	5.07 ± 1.20	0.56	0.78
Q4$_{BMI}$ (*n* = 796)	5.46 ± 1.52	5.45 ± 1.73	0.72	0.75
Triglycerides (mmol/L)				
Men (*n* = 2278)				
Q1$_{BMI}$ (*n* = 561)	1.18 ± 0.56	1.39 ± 1.38	0.015	0.012
Q2$_{BMI}$ (*n* = 578)	1.40 ± 0.83	1.58 ± 0.96	0.016	0.023
Q3$_{BMI}$ (*n* = 583)	1.64 ± 0.91	1.56 ± 0.80	0.36	0.32
Q4$_{BMI}$ (*n* = 556)	1.89 ± 0.96	1.81 ± 0.91	0.49	0.42
Women (*n* = 3223)				
Q1$_{BMI}$ (*n* = 810)	1.03 ± 0.51	1.02 ± 0.43	0.97	0.95
Q2$_{BMI}$ (*n* = 809)	1.13 ± 0.55	1.13 ± 0.51	0.72	0.74
Q3$_{BMI}$ (*n* = 809)	1.30 ± 0.65	1.45 ± 0.98	0.084	0.19
Q4$_{BMI}$ (*n* = 795)	1.54 ± 0.80	1.61 ± 0.84	0.17	0.60

[1] Q1$_{BMI}$-Q4$_{BMI}$ represents quartiles of BMI (in kg/m^2) from the lowest to the highest BMI. Median (interquartile range): Q1$_{BMI}$-Q4$_{BMI}$ for men— Q1$_{BMI}$ = 22.6 (21.5–23.4), Q2$_{BMI}$ = 24.9 (24.4–25.5), Q3$_{BMI}$ = 27.0 (26.5–27.6), and Q4$_{BMI}$ = 30.1 (29.1–31.6); Q1$_{BMI}$-Q4$_{BMI}$ for women— Q1$_{BMI}$ = 21.1 (20.2–22.0), Q2$_{BMI}$ = 23.7 (23.2–24.2), Q3$_{BMI}$ = 26.1 (25.5–26.9), and Q4$_{BMI}$ = 30.5 (28.9–32.8).
[2] A *t* test was used to analyze the data.
[3] P values in the logistic regression were adjusted for age and physical activity.
[4] Mean ± SD.

significant difference according to genotype within any other quartile of BMI (Table 6). In women, carrying of the *T* allele was neither associated with a higher prevalence of diabetes nor with an altered glucose concentration in any of the BMI quartiles (Tables 5 and 6).

Using clinical cutoff levels in the entire sample of men for normal weight (*n* = 871), overweight (*n* = 1407), and obesity (*n* = 283) showed that the OR for diabetes in men carrying the *T* allele compared with carriers of the *CC* genotype was 0.88 (95% CI: 0.48, 1.62; *P* = 0.69) among normal-weight subjects, 1.51 (95% CI: 1.08, 2.13; *P* = 0.02) among overweight subjects, and 1.71 (95% CI: 0.89, 3.31; *P* = 0.11) among the relatively small sample of obese men.

rs1042615 In relation to triglycerides after stratification for fat intake and BMI

Finally, we tested whether the finding of relatively lower triglyceride concentrations in carriers of the rs1042615 *T* allele as compared with carriers of the *CC* genotype was dependent on strata of fat intake or BMI. Because the rs1042615 *T* allele was associated with diabetes in individuals within Q4$_{Fat}$, and diabetes per se is strongly associated with elevated triglyceride concentrations, these analyses were performed in nondiabetic participants only.

Among nondiabetic men there was no consistent association between the *T* allele and low triglyceride concentrations in any of the fat quartiles (Q1$_{Fat}$-Q4$_{Fat}$) (Table 4). In contrast, nondiabetic women within Q4$_{Fat}$ carrying the *T* allele had significantly lower triglyceride concentrations than carriers of the *CC* genotype. This association was no longer significant after adjustment for age, BMI, and physical activity (Table 4). No association was observed between the *T* allele and triglyceride concentrations in women in Q1$_{Fat}$-Q3$_{Fat}$ (Table 4). Finally, no association was observed between the rs1042615 *T* allele and

triglyceride concentrations among either men or women in the upper quartile of BMI (Table 6).

DISCUSSION

The key findings of the present study are that common genetic variation of the *AVPR1A* gene (*T* allele of rs1042615) is associated with slightly higher fasting glucose and lower triglyceride concentrations in a large population-based sample. In addition, the *T* allele was significantly associated with new-onset diabetes exclusively within the highest quartiles of fat intake and BMI, respectively—a finding driven by the male subset of the population and supported by interaction analyses. The *T* allele was associated with lower triglycerides, primarily in the highest quartile of fat intake; however, the association was not significant in the highest quartile of BMI.

Our hypothesis that common human genetic variation may lead to a phenotype similar to that of the V1aR$^{-/-}$ mouse was supported by many of our findings. Male V1aR$^{-/-}$ mice have higher fasting glucose concentrations than WT mice, but they do not develop diabetes, which is in accordance with our findings regarding the rs1042615 polymorphism in humans at the population level.

When fed a high-fat diet, male V1aR$^{-/-}$ mice were more prone to develop overt diabetes and obesity than the WT mice. We attempted to mimic the environmental background of a high-

fat diet applied in the mouse model in an epidemiologic setting by testing the genetic association in strata of our population according to fat intake and BMI. The rs1042615 T allele was significantly associated with diabetes exclusively in the top quartile of fat intake and BMI, respectively. These results were also in accordance with previous findings in the mouse model.

To minimize the risk of over- and underreporting of fat intake, quartiles were based on the residuals of the regression line between fat intake and total energy intake. The median (interquartile range) of fat intake expressed as kcal/d in subjects without known diabetes in the top quartile of fat intake was 1161 (946–1439) for men and 871 (711–1059) for women.

Phenotype-dependent discrepancies between reported current dietary intakes and actual dietary patterns on a long-term retrospective basis may naturally be the case in patients with diabetes. Therefore, we excluded cases with known diabetes (positive self-report of diabetes or use of antidiabetic drugs) in the genotype-phenotype analyses in strata of reported fat intake, which left for analysis only subjects with diabetes diagnosed at the MDC screening event. Furthermore, in a subanalysis of males in Q4$_{Fat}$, excluding all subjects who reported a history of substantial change in their dietary habits ($n = 1666$), the association between rs1042615 and diabetes was stronger (OR: 2.46; 95% CI: 1.05, 5.72; $P = 0.037$).

Given the fact that the knockout mice developed diabetes on the basis of either a high fat intake or obesity or both, we complemented the data derived from stratification of fat intake with that of BMI. The fact that we observed similar results in men in Q1$_{BMI}$-Q4$_{BMI}$ as in those in Q1$_{Fat}$-Q4$_{Fat}$ [ie, the rs1042615 T allele was associated with diabetes exclusively in cases of a high fat intake (Q4$_{Fat}$) and high BMI (Q4$_{BMI}$), respectively] supports our conclusion that the rs1042615 T allele results in a phenotype similar to that observed in male V1aR$^{-/-}$ mice.

Although the association of rs1042615 with glucose concentrations and diabetes in both nonstratified analyses and analyses stratified for fat intake and BMI, as well as the association with triglycerides in nonstratified analysis, were concordant with previous findings in the V1aR$^{-/-}$ mouse, no study has investigated whether triglyceride concentrations differ between V1aR$^{-/-}$ and WT mice in cases of a high fat intake. In humans, we found that the difference in triglycerides between nondiabetic carriers and noncarriers of the rs1042615 T allele was driven by subjects in the top quartile of fat intake, primarily women. However, there was only a borderline significant difference after multivariate adjustment including BMI, and in the top quartile of BMI we observed no difference in triglycerides according to genotype. Thus, although there was an association between rs1042615 and lower triglycerides in the sample as a whole as well as in nondiabetic females in the top quartile of fat intake, the lack of data from the V1aR$^{-/-}$ mice and nonsignificant findings in multivariate-adjusted and BMI-stratified analyses in humans makes it difficult to conclude whether the association between the rs1042615 T allele and lower triglyceride concentrations is enhanced by a high fat intake.

Studies of V1aR$^{-/-}$ and WT mice have shown that the relatively lower concentrations of triglycerides in V1aR$^{-/-}$ mice are caused by enhanced lipolysis that is at least partially a consequence of increased sensitivity of isoproterenol-mediated lipolysis and resistance to insulin-mediated inhibition of lipolysis. As a consequence, glycerol concentrations were increased in the V1aR$^{-/-}$ mouse; however, free fatty acids were reduced because of a simultaneous enhancement of β-oxidation (9). Because the V1aR$^{-/-}$ mouse is glucose intolerant and resistant to insulin-mediated glucose uptake, it can be speculated its hypermetabolism of fat is a compensatory mechanism for the reduced capacity to use glucose as a source of energy. In contrast with V1aR$^{-/-}$ mice, humans with insulin resistance and glucose intolerance usually have high concentrations of triglycerides. We speculate that the cause of relatively higher fasting glucose and lower fasting triglyceride concentrations seen in human carriers of the rs1042615 T allele may have an etiology similar to that of V1aR$^{-/-}$ mice, ie, preferential metabolism of fat instead of glucose as a consequence of reduced expression of the V1a receptor. However, it is important to remember that the regulation of glucose and fat metabolism has a multifactorial and polygenic nature. Thus, it is logical that the association between the rs1042615 T allele and elevations in glucose and reductions in triglycerides is small. Still, the uncoupling of the usual direct relation between glucose and triglycerides is interesting for the understanding of the complex pathophysiology of type 2 diabetes and its related alterations in blood lipids. In fact, similar to the AVPR1A polymorphism, the first type 2 diabetes susceptibility gene to be discovered—transcription factor 7-like 2 (TCF7L2)—is also associated with relatively lower circulating concentrations of triglycerides (18). A greater understanding of how multiple diabetes susceptibility genes interact, and their net effect on glucose and lipid metabolism, may contribute with clues that are important to the development of novel antidiabetic agents.

We do acknowledge a number of limitations of our study. The cross-sectional nature of our phenotypic and dietary data may lead to bias caused by the relation between diabetes and dietary patterns. Although we attempted to eliminate such bias by excluding patients with known diabetes in the analyses stratified by fat intake, these findings need to be confirmed in prospective studies. Furthermore, the significance levels for phenotypic associations with the rs1042615 T allele are relatively modest in relation to the number of tests that were performed. On the other hand, the phenotypes that we tested in relation to genetic AVPR1A variation were all prespecified and defined by previous findings in V1aR$^{-/-}$ mice (8, 9). Furthermore, the 4 SNPs studied were selected according to HapMap data (www.HapMap.org) to capture the maximum of common genetic variation in the AVPR1A gene. Although the rs1042615 T allele was associated with a range of metabolic features similar to those seen in V1aR$^{-/-}$ mice, we had no prior hypothesis concerning which of the 4 SNPs would be associated with such phenotypes, emphasizing the importance of replication of the results.

In conclusion, the rs1042615 T allele may be associated with features resembling the phenotype of V1aR$^{-/-}$ mice, including uncoupling of the usual direct relation between glucose and triglycerides and an increase in the prevalence of diabetes in subjects with a high fat intake or who are overweight. These findings may add to the understanding of the complex pathophysiological background of type 2 diabetes and related lipid disturbances.

EW: shared nutritional knowledge and participated in the analytic design. The authors declared no financial or personal conflicts of interests.

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Epidemiology and Prevention

Plasma Copeptin and the Risk of Diabetes Mellitus

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Background—Animal studies suggest that the arginine vasopressin system may play a role in glucose metabolism, but data from humans are limited.

Methods and Results—We analyzed plasma copeptin (copeptin), a stable C-terminal fragment of the arginine vasopressin prohormone. Using baseline and longitudinal data from a Swedish population-based sample (n=4742; mean age, 58 years; 60% women) and multivariable logistic regression, we examined the association of increasing quartiles of copeptin (lowest quartile as reference) with prevalent diabetes mellitus at baseline, insulin resistance (top quartile of fasting plasma insulin among nondiabetic subjects), and incident diabetes mellitus on long-term follow-up. New-onset diabetes mellitus was ascertained through 3 national and regional registers. All models were adjusted for clinical and anthropometric risk factors, cystatin C, and C-reactive protein. In cross-sectional analyses, increasing copeptin was associated with prevalent diabetes mellitus ($P=0.04$) and insulin resistance ($P<0.001$). During 12.6 years of follow-up, 174 subjects (4%) developed new-onset diabetes mellitus. The odds of developing diabetes mellitus increased across increasing quartiles of copeptin, even after additional adjustment for baseline fasting glucose and insulin (adjusted odds ratios, 1.0, 1.37, 1.79, and 2.09; P for trend=0.004). The association with incident diabetes mellitus remained significant in analyses restricted to subjects with fasting whole blood glucose <5.4 mmol/L at baseline (adjusted odds ratios, 1.0, 1.80, 1.92, and 3.48; $P=0.001$).

Conclusions—Elevated copeptin predicts increased risk for diabetes mellitus independently of established clinical risk factors, including fasting glucose and insulin. These findings could have implications for risk assessment, novel antidiabetic treatments, and metabolic side effects from arginine vasopressin system modulation. (***Circulation***. 2010;121:2102-2108.)

Key Words: arginine vasopressin ■ copeptin ■ diabetes mellitus ■ epidemiology ■ risk factors

Diabetes mellitus is one of the major risk factors for coronary heart disease and congestive heart failure, but the causes of this interaction are not completely understood. One possible link is that hormones implicated in cardiac diseases may play a role in the development of diabetes mellitus. Arginine vasopressin (AVP), also known as antidiuretic hormone, is released from the pituitary gland in conditions of high plasma osmolality, low plasma volume, and low blood pressure. Plasma copeptin, a marker of vasopressin level, is elevated in myocardial infarction[1] and predicts prognosis in patients who develop heart failure after myocardial infarction.[2] Interestingly, recent findings also indicate a cross-sectional association between plasma vasopressin and insulin resistance.[3]

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AVP binds to 3 different receptors (V1aR, V1bR, and V2R). V1aR is widely expressed,[4] whereas V1bR is expressed more specifically in the pituitary gland and pancreas and V2R in the renal collecting ducts.[5–7] The antidiuretic effect of AVP is mediated through V2R, and pharmacological blockade of V2R has been used to treat hyponatremia[8] and heart failure.[9] The prothrombotic and vasoconstrictor effects of AVP are mediated primarily through V1aR,[10,11] and AVP agonists have been used

to treat bleeding and hypotensive disorders.[12,13] In addition, AVP action has been linked to liver glycogenolysis (V1aR) and insulin and glucagon secretion (V1bR).[14,15] Furthermore, AVP stimulates pituitary adrenocorticotropin hormone release (V1bR).[16] This effect has been reported to be resistant to glucocorticoid feedback, suggesting crosstalk between the AVP and hypothalamic-pituitary-adrenalsystems that could be of relevance for diabetes development.[17]

Previous studies in humans and animals have suggested a role for the AVP system in glucose homeostasis, insulin resistance, and diabetes mellitus. In patients with poorly controlled diabetes mellitus, plasma AVP is markedly elevated,[18] and in healthy subjects, AVP infusion leads to increased blood glucose levels.[19] Mice lacking V1aR display impaired glucose tolerance, insulin resistance, and elevated AVP levels[20]; mice that lack V1bR have the opposite phenotype of lower fasting plasma glucose and increased insulin sensitivity.[21] These findings suggest a model in which impaired signaling through V1aR leads to elevated levels of AVP, which in turn stimulates V1bR and contributes to insulin resistance and the development of diabetes mellitus.

There are major concerns regarding the reliability of AVP measurements in plasma because AVP is an unstable molecule both in vivo and ex vivo, is cleared rapidly from plasma, and is largely attached to platelets in the circulation. Copeptin is a cleavage product of the C-terminal part of the AVP precursor that is produced in equimolar amounts with AVP, a process similar to the generation of insulin and C peptide. In contrast to AVP, copeptin is stable, has a long half-life, and is not bound to platelets. Therefore, copeptin is found in considerably higher concentrations in plasma than AVP.[22] Thus, using a validated sandwich assay to measure copeptin in plasma (copeptin), we tested the hypothesis that elevated copeptin is related cross-sectionally to prevalent diabetes mellitus and insulin resistance and longitudinally to increased risk of new-onset diabetes mellitus at the population level.

Methods

Subjects

The Malmö Diet and Cancer study (MDC) is a population-based prospective cohort consisting of 28 449 persons surveyed in 1991 to 1996.[23] From this cohort, 6103 persons were randomly selected to be studied for the epidemiology of carotid artery disease; this sample is referred to as the MDC cardiovascular cohort (MDC-CC). Fasting plasma samples were obtained in 5405 subjects in the MDC-CC.[24] Of those, complete data on covariates, including risk factors for diabetes mellitus, potential confounders, and copeptin, were available in 4742 individuals.

All analyses in plasma and whole blood were performed on overnight fasting samples. Analyses of fasting whole-blood glucose (FBG), plasma lipids, and insulin were carried out at the time of baseline examination at the Department of Clinical Chemistry, Malmö University Hospital, which is attached to a national standardization and quality control system. The limit of detection for insulin was 3 mIU/L, and the intra-assay and interassay coefficients of variation were 5% and 8%, respectively.[25] FBG was measured in whole-blood glucose by a hexokinase-glucose-6-phosphate dehydrogenase method. In fasting plasma samples, we measured copeptin using a commercially available assay in the chemiluminescence/coated-tube format (BRAHMS AG, Hennigsdorf, Germany) as described previously.[26] C-reactive protein was measured by a high-sensitivity assay (Tina-Quant C-reactive protein, Roche Diagnostics, Basel, Switzerland). Cystatin C was mea-

sured with a particle-enhanced immunonephelometric assay (N Latex Cystatin C, Dade Behring, Ill).

Blood pressure was measured with a mercury-column sphygmomanometer after 10 minutes of rest in the supine position. Cigarette smoking was elicited by a self-administered questionnaire, with current cigarette smoking defined as any smoking within the past year. Prevalent cardiovascular disease was defined as the occurrence of myocardial infarction or stroke before the baseline examination obtained through national registers as described previously.[24] Family history of diabetes mellitus was obtained by a questionnaire and defined as known diabetes mellitus in at least 1 first-degree relative.

Diabetes mellitus at baseline was defined as self-report of a physician diagnosis or use of diabetes medication or a FBG of ≥6.1 mmol/L (corresponding to fasting plasma glucose concentration of ≥7.0 mmol/L). Subjects who belonged to the top quartile of fasting insulin concentration in the segment of the population without diabetes mellitus were defined as having insulin resistance, as proposed by the European Group for the Study of Insulin Resistance.[27] New-onset diabetes mellitus diagnosed after the baseline examination until December 2005 (mean follow-up time, 12.6 years) was assessed in subjects free of diabetes mellitus at baseline by 3 registers: the Malmö HbA$_{1c}$ register (MHR), the nationwide Swedish National Diabetes Register (NDR),[28] and the regional Diabetes 2000 register of the Scania region, of which Malmö is the largest city.[29] NDR and the Diabetes 2000 register required a physician diagnosis according to established diagnostic criteria (fasting plasma glucose concentration of ≥7.0 mmol/L, which corresponds to an FBG concentration of ≥6.1 mmol/L, measured on 2 different occasions).

The MHR at the Department of Clinical Chemistry, Malmö University Hospital, analyzed and cataloged all hemoglobin A$_{1c}$ (HbA$_{1c}$) samples taken in institutional and noninstitutional care in the greater Malmö area from 1988 on. Individuals who had at least 2 HbA$_{1c}$ recordings ≥6.0% in the MHR with the Swedish Mono-S standardization system (corresponding to 7.0% according to the US National Glycohemoglobin Standardization Program) after the MDC-CC baseline examination were defined as incident diabetes cases. Furthermore, subjects who were registered in the NDR or the Diabetes 2000 register after the MDC-CC baseline examination were defined as incident diabetes cases.

Subjects who were free of diabetes mellitus at the baseline examination in the MDC-CC, defined by lack of self-reported history of physician-diagnosed diabetes mellitus, use of diabetes medication, and FBG at the baseline examination of ≥6.1 mmol/L, but were registered as diabetes cases any time after their baseline examination in the MDC-CC until December 2005 were classified as having new-onset diabetes mellitus. With these criteria, 174 subjects had new-onset diabetes mellitus during follow-up. Of these, more than half (52%) were identified as new-onset diabetes cases via at least 2 of the 3 registers.

A subset (n=887) of the MDC-CC was reinvestigated after a mean of 6.6 years.[30] Of subjects free of diabetes mellitus or without impaired fasting glucose (IFG) at baseline, complete data on covariates, including risk factors for diabetes mellitus, potential confounders, and copeptin, were available in 745 and 620 individuals, respectively. Diabetes development was defined as FBG ≥6.1 mmol/L at the time of follow-up.

The study protocols were approved by the ethics committee of Lund University. All participants provided written informed consent.

Statistical Analyses

SPSS statistical software (version 14.0, SPSS Inc, Chicago, Ill) was used for all analyses except calculation of the C statistics, which was performed with Stata software version 8.0 (Stata Corp, College Station, Tex). Group-wise differences in continuous variables at baseline were tested with the Student t test and reported as mean±SD if normally distributed and with the Mann-Whitney test and reported as medians and interquartile ranges if not normally distributed. Variables that were not normally distributed were transformed with the natural logarithm when analyzed as continuous variables. Differences in dichotomous variables were tested with χ^2 tests. The P value for linear trend in FBG and insulin levels over quartiles of copeptin in subjects without diabetes mellitus was assessed with linear regression. We used crude and multivariable-

Table 1.   Baseline Characteristics of Subjects With Normal Fasting Glucose, With IFG, and With Diabetes Mellitus

	NFG Subjects (n=3702)	IFG Subjects (Excluding DM) (n=675)	DM Subjects (n=365)	P, NFG vs DM	P, IFG vs DM
Age, y	57.3±5.9	57.9±5.8	59.5±5.5	<0.001	<0.001
Men, %	38.3	53.9	56.2	<0.001	0.49
FBG, mmol/L	4.8±0.34	5.6±0.19	8.1±3.0	<0.001	<0.001
Triglycerides, mmol/L*	1.14 (0.86–1.58)	1.34 (1.00–1.86)	1.64 (1.13–2.33)	<0.001	<0.001
Systolic BP, mm Hg	139±19	146±19	150±20	<0.001	0.001
Diastolic BP, mm Hg	86.1±9.3	89±9.6	90±9.5	<0.001	0.04
AHT, %	15.7	20.9	37.3	<0.001	<0.001
BMI, kg/m²	25.2±3.6	27.0±3.9	28.7±4.5	<0.001	<0.001
Waist, cm	81.5±12	88.8±12	94.4±13	<0.001	<0.001
Waist-to-hip ratio	0.84±0.09	0.88±0.09	0.91±0.09	<0.001	<0.001
HDL, mmol/L	1.42±0.37	1.31±0.35	1.23±0.35	<0.001	0.001
LDL, mmol/L	4.1±0.98	4.3±1.0	4.2±1.0	0.08	0.23
Cystatin C, mg/L	0.771±0.144	0.787±0.137	0.809±0.193	<0.001	0.04
Copeptin, pmol/L*	5.14 (3.20–8.14)	5.77 (3.54–9.32)	6.90 (4.32–10.7)	<0.001	0.01
Insulin, mU/L*	6.0 (4.0–9.0)	8.0 (6.0–12)	12 (7.0–18)	<0.001	<0.001
CRP, mg/L*	1.3 (0.7–2.8)	1.7 (0.8–3.4)	2.3 (1.3–4.5)	<0.001	<0.001
Current smoker, %	25.9	29.1	24.4	0.50	0.12
Family history of DM, %	3.1	2.1	3.0	0.90	0.35
Previous CVD, %	2.1	2.8	3.8	0.01	0.37

NFG indicates normal fasting glucose; DM, diabetes mellitus; BP, blood pressure; AHT, antihypertensive treatment; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; and previous CVD, cardiovascular disease present before baseline examination. Continuous variables are given as mean±SD unless otherwise specified.

*Median (interquartile range).

adjusted logistic regression to test whether increasing quartiles of copeptin (quartiles 2 through 4 compared with quartile 1) were related to prevalent diabetes mellitus at baseline in the entire cohort (n=4742). In subjects without diabetes mellitus at baseline (n=4377), crude regression and multivariable logistic regression were used to test whether increasing quartiles of copeptin were related to insulin resistance and to new-onset diabetes mellitus. In addition, the relationship between increasing quartiles of copeptin and risk of new-onset diabetes mellitus was tested in subjects without IFG at the baseline examination (fasting plasma glucose <6.1 mmol/L corresponding to an FBG <5.4 mmol/L; n=3702). Finally, multivariable logistic regression was used to test the relationship between increasing quartiles of copeptin and incident diabetes mellitus on the basis of FBG thresholds in the subset of MDC-CC that was reinvestigated. Data from logistic regression analyses are expressed as odds ratios (ORs) and 95% confidence intervals (CIs). A 2-sided value of $P<0.05$ was considered statistically significant.

To assess the sensitivity and specificity of copeptin in predicting new-onset diabetes mellitus in addition to classic diabetes predictors, we compared the area under the receiver-operating characteristic curves using both a personal model (age, gender, body mass index, and family history of diabetes mellitus) and a clinical model (personal model plus systolic blood pressure, triglycerides, high-density lipoprotein, waist circumference, and FBG) as previously proposed[31] with and without copeptin in each of the 2 models. The integrated discrimination improvement was calculated as described previously.[32]

We chose logistic regression in our primary analyses rather than Cox regression (time to event) because the diagnosis of diabetes mellitus commonly occurs years after the actual onset of diabetes mellitus.[33] In secondary analyses, we performed Cox regression models with the time of the diabetes event defined as the date of the first HbA$_{1c}$ value ≥6% in MHR, registration in the Diabetes 2000 register, or registration in NDR. C statistics based on the Cox regressions were calculated as described previously.[34]

Results

Baseline characteristics according to diabetes status are shown in Table 1. A total of 365 individuals had diabetes mellitus at the baseline examination; 29% had a history of a physician-diagnosis of diabetes mellitus or diabetes treatment; and 71% had an FBG ≥6.1 mmol/L without an accompanying physician diagnosis.

At the baseline examination, copeptin concentrations were higher among individuals with diabetes mellitus compared with subjects without diabetes mellitus (Table 1). Among nondiabetic subjects at baseline, the Pearson correlations between copeptin and potential confounders, ie, body mass index, insulin, and glucose, were 0.091, 0.12, and 0.10, respectively ($P<0.001$ for all). Increasing quartile of copeptin was associated with an increased odds of diabetes mellitus in a crude model and after multivariable adjustment for all baseline covariates that differed between diabetes patients and subjects without diabetes mellitus (Table 1) except for FBG and fasting plasma insulin concentration (model 1 adjustment) (Table 2). In the segment of the population free of diabetes mellitus, ie, in subjects with a range of glucose values that should not affect copeptin through osmolality, copeptin was positively associated with FBG (the Figure). Additionally, copeptin was positively associated with plasma concentration of insulin (the Figure). The odds of insulin resistance increased with increasing copeptin both in crude analysis and after adjustment for model 1 covariates and FBG (Table 2). The association between increasing quartiles of copeptin and the homeostasis model assessment insulin re-

Table 2.   Prevalent Diabetes Mellitus and Insulin Resistance in Relation to Quartiles of Baseline Copeptin at Baseline

Dependent Variable	OR (95% CI)			*P* (Test for Linear Trend)
	Copeptin Q2 vs Q1	Copeptin Q3 vs Q1	Copeptin Q4 vs Q1	
Diabetes*				
Crude	1.44 (1.00–2.07)	1.92 (1.36–2.71)	2.83 (2.04–3.93)	<0.001
Adjusted†	1.19 (0.81–1.75)	1.39 (0.96–2.01)	1.45 (1.00–2.11)	0.04
Hyperinsulinemia‡				
Crude	1.30 (1.06–1.60)	1.53 (1.25–1.87)	2.34 (1.93–2.85)	<0.001
Adjusted§	1.19 (0.94–1.51)	1.25 (0.99–1.59)	1.61 (1.26–2.06)	<0.001

Q indicates quartile.

*Analysis of prevalence of diabetes mellitus (n=365) in the entire cohort (n=4742).

†Adjusted for age, sex, high-density lipoprotein, triglycerides, blood pressure, antihypertensive treatment, body mass index, waist, waist-to-hip ratio, cystatin C, C-reactive protein, and previous cardiovascular disease (model 1).

‡Analysis of hyperinsulinemia (highest quartile of fasting plasma insulin concentration among nondiabetic subjects) among nondiabetic subjects (n=4377).

§Adjusted for model 1 and FBG.

sistance index (the product of fasting insulin and FBG) was similar (data not shown).

Among participants free of diabetes mellitus at baseline (n=4377), 174 subjects developed new-onset diabetes mellitus (Table 3) according to the 3 different registers. MHR captured 106 subjects, whereas the Diabetes 2000 registry and NDR captured 110 and 76 subjects, respectively. Of the 106 diabetes cases diagnosed by MHR, 71% (n=75) were also diagnosed in Diabetes 2000 and/or NDR.

When the baseline sample was further restricted to subjects without IFG (n=3702), 79 subjects developed new-onset diabetes mellitus during follow-up. Continuous copeptin concentration at baseline was significantly higher in subjects who subsequently developed new-onset diabetes mellitus compared with those who did not, regardless of whether subjects with IFG at baseline were included (Table 3). The likelihood of developing new-onset diabetes mellitus increased with increasing copeptin quartiles in crude logistic regression analysis and after multivariate adjustment (model 1 covariates and fasting insulin, FBG, smoking, diabetes heredity, and low-density lipoprotein) in both subjects free of diabetes mellitus at baseline (*P* for trend <0.001, crude; *P*=0.004, multivariable adjusted) and subjects free of IFG at baseline (*P*<0.001, crude; *P*=0.001, multivariable-adjusted; Table 4).
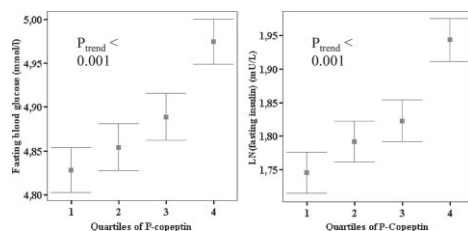
Figure. Fasting concentrations of glucose in whole blood and insulin in plasma (the latter transformed using the natural logarithm), expressed as mean with 95% CIs, in nondiabetic subjects belonging to quartiles of increasing copeptin (n=4377). *P* for trend <0.001 in both analyses.

In subjects free of diabetes mellitus at baseline, the area under the receiver-operating characteristic curve increased from 0.694 to 0.710 (*P*=0.08) when copeptin was added to the personal model and from 0.832 to 0.841 (*P*=0.007) when copeptin was added to the clinical model of diabetes prediction. In subjects free of IFG at baseline, the area under the receiver-operating characteristic curve increased from 0.663 to 0.713 (*P*=0.03) and from 0.783 to 0.805 (*P*=0.04) when copeptin was added to the personal model and clinical model for diabetes prediction, respectively. Integrated discrimination improvement was significantly improved by adding copeptin to the personal model for diabetes prediction in both nondiabetic subjects (*P*=0.01) and non-IFG subjects (*P*=0.02) but nonsignificant when added to the clinical model (*P*=0.35 in nondiabetic individuals, *P*=0.09 in non-IFG individuals).

To validate the association between copeptin at baseline and the register-based diabetes end point, we assessed the multivariate-adjusted relationship between copeptin and diabetes development on the basis of elevated FBG in a subset of the MDC-CC that was reinvestigated after 6.6 years. Among those free of diabetes mellitus at baseline, 63 subjects had an elevated FBG during follow-up. Increasing quartile of copeptin was associated with new-onset diabetes mellitus using this criterion both in subjects free of diabetes mellitus at baseline (OR, 1.42; 95% CI, 1.04 to 1.94; *P*=0.03) and in subjects free of IFG at baseline (OR, 1.67; 95% CI, 1.07 to 2.63; *P*=0.03) after full adjustment (model 1 covariates and fasting insulin, FBG, smoking, diabetes heredity, and low-density lipoprotein), comparable to the findings reported in Table 4.

Finally, Cox regression models were performed to exclude potential bias related to interindividual differences in follow-up time in the logistic regressions models, and the results were unchanged (Table I in the online-only Data Supplement). C statistics derived from Cox regression models were similar to those from logistic regression models. With the addition of copeptin, the C statistic from Cox regression models increased in subjects without diabetes mellitus at baseline from 0.702 to 0.718 in the personal model and from 0.835 to 0.844 in the clinical model. When analyses were restricted to subjects without IFG at baseline, the C statistic

Table 3. Baseline Characteristics in Subjects Who Did and Did Not Convert to Diabetes Mellitus During Follow-Up

	Nonconverters	Incident Diabetes Mellitus	P
Subjects without diabetes mellitus at baseline (n=4377)			
n	4203	174	
Age, y	57.3±5.9	57.9±5.7	0.26
Men, %	38.9	45.4	0.09
FBG, mmol/L	4.9±0.44	5.4±0.44	<0.001
Triglycerides, mmol/L*	1.11 (0.84–1.51)	1.46 (1.05–1.96)	<0.001
Systolic BP, mm Hg	140±19	146±19	<0.001
Diastolic BP, mm Hg	86±9.3	90±10	<0.001
AHT, %	14.2	27.6	<0.001
BMI, kg/m^2	25.4±3.6	28.2±4.7	<0.001
Waist, cm	82.2±12	91.5±14	<0.001
Waist-to-hip ratio	0.84±0.09	0.89±0.10	<0.001
HDL, mmol/L	1.41±0.37	1.25±0.33	<0.001
LDL, mmol/L	4.2±0.98	4.3±1.0	0.06
Cystatin C, mg/L	0.77±0.14	0.82±0.21	<0.001
Copeptin, pmol/L*	4.98 (3.09–7.84)	6.35 (4.05–9.88)	<0.001
Insulin, mU/L*	6.0 (4.0–9.0)	9.0 (6.0–13)	<0.001
CRP, mg/L*	1.2 (0.6–2.6)	2.1 (0.9–4.1)	<0.001
Current smoker, %	26.4	29.5	0.37
Family history of diabetes mellitus, %	2.8	6.9	0.002
Previous CVD, %	2.0	2.3	0.80
Subjects without IFG at baseline (n=3702)			
n	3623	79	
Age, y	57.2±5.9	58.1±5.4	0.20
Men, %	36.5	38.0	0.79
FBG, mmol/L	4.7±0.34	5.0±0.28	<0.001
Triglycerides, mmol/L*	1.08 (0.82–1.47)	1.34 (1.06–1.92)	<0.001
Systolic BP, mm Hg	139±19	146±19	0.001
Diastolic BP, mm Hg	86±9.2	89±9.5	0.004
AHT, %	13.4	24.1	0.006
BMI, kg/m^2	25.2±3.6	27.5±4.9	<0.001
Waist, cm	81.3±12	88.7±14	<0.001
Waist-to-hip ratio	0.84±0.09	0.87±0.09	<0.001
HDL, mmol/L	1.43±0.37	1.27±0.32	<0.001
LDL, mmol/L	4.1±0.98	4.1±0.93	0.99
Cystatin C, mg/L	0.77±0.14	0.83±0.27	<0.001
Copeptin, pmol/L*	4.90 (3.03–7.65)	6.74 (4.44–10.9)	0.001
Insulin, mU/L*	6.0 (4.0–8.0)	8.0 (6.0–11)	<0.001
CRP, mg/L*	1.2 (0.6–2.4)	2.4 (1.0–4.1)	<0.001
Current smoker, %	26.0	30.4	0.38
Family history of diabetes mellitus, %	3.0	7.6	0.02
Previous CVD, %	1.9	2.5	0.67

Abbreviations as in Table 1. Continuous variables are given as mean±SD unless otherwise specified.

*Median (interquartile range).

increased from 0.671 to 0.721 in the personal model and from 0.790 to 0.811 in the clinical model.

Discussion

The key finding of our study is that copeptin predicts the development of diabetes mellitus independently of renal function and a broad range of diabetes risk factors at baseline, including FBG and fasting insulin. The association between baseline copeptin and incident diabetes mellitus was particularly strong in those individuals free of IFG at baseline.

As expected, FBG was the strongest risk factor for new-onset diabetes mellitus. Each 1-mmol/L increase in FBG at baseline increased the risk of future diabetes mellitus with an OR of 11.4 (95% CI, 7.4 to 17.5) in the fully adjusted model. Nonetheless, after accounting for FBG and all other available diabetes risk factors, individuals in the top quartile of copeptin had a 2- to 3-fold excess risk of developing diabetes mellitus compared with those in the lowest quartile of copeptin. In subjects without IFG, there was a significant improvement in the area under the receiver-operating characteristic curve when copeptin was added to classic diabetes risk factors, consistent with an improvement in discrimination. Additionally, inclusion of copeptin was associated with a significant integrated discrimination improvement compared with the personal model and a borderline significant integrated discrimination improvement compared with the clinical model. The improvement in the area under the receiver-operating characteristic curve was smaller when the entire sample, including those with IFG, was considered. This finding may result from FBG being a more powerful predictor of diabetes mellitus at FBG levels near the diagnostic limit of 6.1 mmol/L. Markers that are not part of the diagnostic criteria for diabetes mellitus such as copeptin may better signal diabetes susceptibility earlier in the prediabetes state. Novel risk markers as screening tools for future diabetes risk could be particularly useful in individuals with normal FBG, who are likely to be less closely monitored than individuals with IFG. Thus, our findings suggest that copeptin could provide incremental information for the prediction of diabetes mellitus.

Apart from any implications for diabetes prediction, our data support a role for the AVP system in the pathophysiology of diabetes mellitus. Animal studies have shown that mice lacking V1aR display elevated levels of AVP, glucose intolerance, and insulin resistance, whereas mice lacking V1bR have the opposite phenotype of lower FBG and improved insulin sensitivity.[20,21] From these animal data and our clinical findings, it can be speculated that elevated AVP, as a consequence of AVP resistance at the level of V1aR or elsewhere, could contribute to insulin resistance and diabetes mellitus through stimulation of V1bR. In fact, pharmacological blockade of V2R, a potent stimulus of increased AVP secretion,[35] was associated with a 5% incidence of hyperglycemic events during 30 days of tolvaptan treatment in patients with hyponatremia compared with 1% in control subjects, although the numbers were small (12 of 223 versus 2 of 220).[8] Further studies on the potential metabolic effects of pharmacological manipulation of the AVP system may be warranted.

The diabetes incidence in our study may appear low but was comparable to that in a previous Swedish study.[36] Still, several potential factors could result in a lower-than-expected

Table 4. New-Onset Diabetes Mellitus in Relation to Quartiles of Baseline Copeptin

	OR (95% CI)			
Dependent Variable	Copeptin Q2 vs Q1	Copeptin Q3 vs Q1	Copeptin Q4 vs Q1	*P* (Test for Linear Trend)
Incident diabetes mellitus among non–diabetes mellitus*				
Crude	1.28 (0.76–2.15)	1.94 (1.19–3.14)	2.64 (1.66–4.19)	<0.001
Adjusted†	1.37 (0.78–2.39)	1.79 (1.06–3.05)	2.09 (1.23–3.56)	0.004
Incident diabetes mellitus among non-IFG‡				
Crude	1.85 (0.81–4.20)	2.12 (0.95–4.73)	4.56 (2.18–9.52)	<0.001
Adjusted†	1.80 (0.78–4.16)	1.92 (0.84–4.38)	3.48 (1.58–7.65)	0.001

*Subjects who developed diabetes mellitus during follow-up (n=174) among subjects without diabetes mellitus at baseline (n=4377).

†Adjusted for age, sex, high-density lipoprotein, triglycerides, blood pressure, antihypertensive treatment, body mass index, waist, waist-to-hip ratio, cystatin C, C-reactive protein, prevalent cardiovascular disease, smoking, family history of diabetes mellitus, low-density lipoprotein, FBG, and fasting insulin.

‡Subjects who developed diabetes mellitus during follow-up (n=79) among subjects without IFG at baseline (n=3702).

number of new-onset diabetes cases in our study. First, because the participation rate was only 40%, the MDC population is healthier than the background population.[37] Second, the diabetes incidence in the 3 registers is based on people who actively seek health care, leading to a lower incidence than observed in studies that directly screen for diabetes mellitus by measuring FBG. Third, at baseline, the classification of prevalent diabetes mellitus was based solely on FBG ≥6.1 mmol/L in as many as 71% of diabetes patients. Excluding these previously unrecognized diabetes cases from follow-up minimizes the risk that we have incorrectly classified some subjects as incident, instead of prevalent, cases of diabetes mellitus. Finally, because of incomplete register coverage, our study may have missed new-onset cases of diabetes mellitus that were in fact diagnosed within the healthcare system. These factors would be expected to bias our results toward the null. On the other hand, given the strict HbA$_{1c}$ criterion for new-onset diabetes mellitus in the MHR and the fact that the NDR and the Diabetes 2000 register require a physician diagnosis according to established diagnostic criteria,[28,29] those cases classified as new onset in our study are unlikely to be misclassified. The validity of the register-based new-onset diabetes diagnosis is further supported by most of the well-known risk factors for diabetes mellitus being markedly elevated at the MDC-CC baseline in these subjects compared with those who did not develop diabetes mellitus according to the 3 registers (Table 3). Importantly, these differences were equally or more pronounced when the study population was restricted to subjects free of IFG at baseline, excluding the possibility that our findings regarding established diabetes risk factors and copeptin in relation to new-onset diabetes mellitus were driven solely by subjects who had IFG at the MDC-CC baseline examination. The fact that we were able to reproduce the association between copeptin and the register-based diabetes end point using an FBG-based end point in a subset of MDC-CC strongly supports the validity of our findings.

Conclusions

Copeptin predicts diabetes mellitus independently of a broad range of established diabetes risk factors, including fasting levels of glucose and insulin. Our findings suggest a role of the AVP system in the development of diabetes mellitus and may have implications for risk assessment and the development of novel diabetes pharmacotherapy. In addition, our results highlight the possibility that existing therapies that selectively antagonize AVP receptors could have metabolic side effects.

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CLINICAL PERSPECTIVE

Despite the fact that diabetes mellitus is a potent risk factor for cardiovascular morbidity and mortality, therapies focused on lowering plasma glucose have not been convincingly shown to reduce cardiovascular mortality. Thus, identifying drug-modifiable factors other than glucose that link diabetes mellitus to cardiovascular disease is important. Copeptin, a stable fragment of the vasopressin precursor, has been studied in risk prediction in myocardial infarction and congestive heart failure. Vasopressin is known to affect liver glycogenolysis, insulin, and glucagon secretion and pituitary adrenocorticotropin hormone release. Here, we show in a large prospective cohort study that an increased copeptin level in plasma predicts future diabetes mellitus independently of known risk factors. In nondiabetic individuals, the top versus bottom quartile of copeptin the risk of diabetes mellitus was increased >2-fold; among individuals with normal fasting glucose, the risk was increased 3.5-fold. Our data suggest that plasma copeptin may be valuable for diabetes risk assessment, especially in subjects without impaired fasting glucose, and point to a new pharmacologically modifiable system of potential importance in both diabetes mellitus and cardiovascular disease.

SUPPLEMENTAL MATERIAL

Supplemental Table 1. New-onset diabetes in relation to quartiles of baseline copeptin.

Dependent variable		HR* (95% CI) Q2 $_{copeptin}$ vs Q1$_{copeptin}$	HR* (95% CI) Q3 $_{copeptin}$ vs Q1 $_{copeptin}$	HR* (95% CI) Q4 $_{copeptin}$ vs Q1 $_{copeptin}$	P (test for linear trend)
Incident diabetes	crude	1.30 (0.78-2.17)	1.96 (1.22-3.16)	2.70 (1.71-4.25)	<0.001
among non-DM†	adjusted§	1.31 (0.78-2.20)	1.67 (1.02-2.72)	1.88 (1.15-3.05)	0.007
Incident diabetes	crude	1.89 (0.83-4.27)	2.17 (0.98-4.84)	4.68 (2.26-9.72)	<0.001
among non-IFG‡	adjusted§	1.76 (0.77-4.01)	1.86 (0.83-4.18)	3.28 (1.52-7.10)	0.002

*Hazard Ratio.

†Subjects who developed diabetes during follow-up (n=174) among subjects without diabetes at baseline (n=4377).

‡Subjects who developed diabetes during follow-up (n=79) among subjects without impaired fasting glucose at baseline (n=3702).

§Adjusted for age, sex, HDL, triglycerides, blood pressure, antihypertensive treatment, body mass index, waist, waist/hip ratio, cystatin C, CRP and prevalent cardiovascular disease, smoking, family history of diabetes, LDL, FBG and fasting insulin.

III

Plasma Copeptin, A Unifying Factor behind the Metabolic Syndrome

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Context: Arginine vasopressin (AVP) is known to affect liver glycogenolysis, insulin, and glucagon secretion and pituitary ACTH release. We previously showed that high copeptin, the stable C-terminal fragment of AVP prohormone, is independently associated with hyperinsulinemia and future development of diabetes mellitus.

Objective: The objective of the study was to examine whether plasma copeptin is associated with components of the metabolic syndrome (MetS) independently of insulin, diabetes mellitus, and environmental factors.

Design, Setting, and Participants: This was a cross-sectional, population-based sample of 4742 subjects, aged 46–68 yr, 60% women, in Malmö, Sweden.

Main Outcome Measure: Using multivariable logistic and linear regression, plasma copeptin was associated with components of the MetS.

Results: Copeptin quartile (lowest quartile as reference) was, after adjustment for age, sex, insulin, and diabetes mellitus, associated with hypertension (odds ratios 1.04, 1.07, 1.31; $P = 0.004$), abdominal obesity (odds ratios 1.21, 1.16, 1.57; $P = 0.002$), obesity (odds ratios 1.25, 1.15, 1.49; $P = 0.01$), top quartile of c-reactive protein (odds ratios 1.11, 1.13, 1.32; $P = 0.007$), and MetS (adjusted for age and sex only) (odds ratios 1.53, 1.77, 1.86; $P < 0.001$). High copeptin levels were significantly associated with high fat intake, low physical activity, and borderline significantly associated with low socioeconomic status. The association between copeptin and components of the MetS was not affected after adjustment for these environmental factors.

Conclusions: Our data suggest that increased activity of the AVP system is a unifying factor in the MetS and point to a new pharmacologically modifiable system of potential importance in the treatment of MetS and prevention of cardiovascular disease. (**J Clin Endocrinol Metab** 96: 0000–0000, 2011)

The metabolic syndrome (MetS) is a cluster of cardiometabolic risk factors including hypertension, abdominal obesity, dyslipidemia, insulin resistance, and proinflammatory and prothrombotic states. Almost one of four in the adult population is affected, and individuals with the MetS are at essentially twice the risk for cardiovascular disease compared with those without MetS (1). In addition to genetic and environmental factors contributing to each individual MetS component, it has been suggested that there are unifying etiological factors contributing to the entire cluster. Despite the multifactorial etiological background of Mets, knowledge of its underlying causes is a prerequisite for the identification of more specific lifestyle and pharmacological treatment.

Arginine vasopressin (AVP) is released from the pituitary gland in conditions of high plasma osmolality, low plasma

Abbreviations: AVP, Arginine vasopressin; BMI, body mass index; BP, blood pressure; CI, confidence interval; CRP, c-reactive protein; DM, diabetes mellitus; FBG, fasting blood glucose; HDL, high-density lipoprotein; MetS, metabolic syndrome; OR, odds ratio; TG, triglycerides; waist, waist circumference.

volume, and low blood pressure. AVP is involved in diverse physiological functions, including vasoconstriction, platelet aggregation, stimulation of liver glycogenolysis, inhibition of diuresis, modulation of ACTH secretion from the pituitary, and insulin and glucagon secretion from the pancreas. These effects are mediated through three different receptors (V1aR, V1bR, and V2R). The V1aR is widely expressed (2), whereas V1bR is more specifically expressed in the pituitary gland, white adipose tissue, and pancreas and V2R in the renal collecting ducts (3–6). The antidiuretic effect of AVP is mediated through V2R, whereas the prothrombotic and vasoconstrictor effects of AVP are primarily mediated through the V1aR (7, 8).

AVP is an unstable molecule both *in vivo* and *ex vivo*, is rapidly cleared from plasma and is largely attached to platelets in the circulation. Thus, there are concerns regarding the reliability of AVP measurements in plasma. Copeptin, a cleavage product of the C-terminal part of the AVP precursor, is produced stoichiometrically with AVP. Because copeptin has a long half-life and is not bound to platelets, it is found in considerably higher concentrations in plasma than AVP and is easily detected with a validated sandwich assay for measurement of copeptin in plasma (copeptin). Copeptin levels correlate to AVP levels in plasma (9).

Recently we showed that high copeptin is independently associated with hyperinsulinemia and that it predicts future development of diabetes mellitus (DM) (10). Previous findings indicate several links between the AVP system and components of the MetS. A cross-sectional association was found between plasma copeptin and MetS, high waist circumference (waist), systolic blood pressure (BP), DM and triglycerides (TG) after adjustment for body mass index (BMI), sex, and age in a hypertensive population (11). Furthermore, data from humans and animals have suggested involvement of the AVP system in fat metabolism; AVP stimulate production of triglycerides in rat hepatocytes (12) and accordingly, V1aR-deficient mice have low triglycerides compared to wild type (3). AVP exerts diverse actions on BP, including vasoconstriction, volume control, and direct cardiac effects (13).

In the present study, we aimed at expanding our previous findings of strong relationship between copeptin and hyperinsulinemia and DM by testing the hypothesis that elevated copeptin is associated to MetS, hypertension, waist circumference, BMI, c-reactive protein (CRP), high-density lipoprotein (HDL) and TG independently of DM and insulin. Furthermore, we analyzed the links between copeptin and the environmental factors, *e.g.* socioeconomic status, smoking habits, physical activity, alcohol intake, and fat intake, which are all factors known to cluster with components of the MetS (14–18). Finally, we investigated whether any association between compo-

nents of the MetS and copeptin is independent of the environmental factors influencing copeptin levels.

Subjects and Methods

Subjects

The Malmö Diet and Cancer study is a population-based prospective cohort consisting of 28,449 persons surveyed in 1991–1996 (19). From this cohort, 6103 persons were randomly selected to be studied for the epidemiology of carotid artery disease, and this sample is referred to as the Malmö Diet and Cancer cardiovascular cohort. Fasting plasma samples were obtained in 5405 subjects in the Malmö Diet and Cancer cardiovascular cohort (20). Of those, complete data on covariates, including components of the MetS, potential confounders, and copeptin, were available in 4742 individuals.

All analyses in plasma and whole blood were performed in overnight fasting samples. Analyses of fasting plasma lipids, whole-blood glucose [fasting blood glucose (FBG)], and insulin were carried out at the time of baseline examination at the Department of Clinical Chemistry, Skane University Hospital in Malmö, which is attached to a national standardization and quality control system. CRP was measured by a high-sensitivity assay (Tina-quant CRP; Roche Diagnostics, Basel, Switzerland). Copeptin was measured in fasting plasma samples using a commercially available assay in the chemiluminescence/coated tube format (B.R.A.H.M.S. GmbH, Hennigsdorf, Germany) as described previously (21).

According to the National Cholesterol Education Program Adult Treatment Panel III criteria (22), we classified subjects as having the MetS if they had three or more of the following characteristics: waist circumference greater than 102 cm in men and greater than 88 cm in women; fasting FBG 5.4 mmol/liter or greater (corresponding to fasting plasma glucose concentration of 6.1 mmol/liter or greater) or treatment for DM; HDL less than 1.0 mmol/liter in men and less than 1.3 mmol/liter in women; TG 1.7 mmol/liter or greater; and systolic BP 130 mm Hg or greater and/or diastolic BP 85 mm Hg or greater or use of antihypertensive medication.

BP was measured using a mercury-column sphygmomanometer after 10 min of rest in the supine position. Hypertension was defined as baseline systolic BP 140 mm Hg or greater, diastolic BP 90 mm Hg or greater, or use of antihypertensive medication according to a baseline questionnaire or 7-d menu book. DM at baseline was defined as self-report of a physician diagnosis or use of diabetes medication or FBG of 6.1 mmol/liter or greater (corresponding to fasting plasma glucose concentration of 7.0 mmol/liter or greater). Hyperinsulinemia was defined as the top quartile of fasting plasma insulin concentration. Obesity was defined as BMI of 30 kg/m^2 or greater. Abdominal obesity was defined as waist circumference greater than 102 cm in men and greater than 88 cm in women. Subjects who belonged to the top quartile of plasma CRP concentration were defined as having high CRP.

Leisure-time physical activity was assessed on the basis of a list of activities (18 items) adapted from the Minnesota Leisure Time Physical Activity instrument (23). A score was obtained by multiplying the reported amount of minutes per week spent on a specific activity by an activity-specific factor. Based on this score, the population was ranked into quartiles, and low physical activity was defined as the lowest quartile.

Dietary data were collected through a modified diet history methodology described previously (24) consisting of a 7-d menu book that collected information on cooked lunches and dinners and cold beverages (including alcoholic beverages), a 168-item diet questionnaire to obtain information on frequencies and portion sizes of regularly consumed foods, not reported in the menu book (during the past year), and a complementary 1-h interview. During the interview participants were asked to describe food choices, food preparation practices, and portion sizes of the foods reported in the menu book (using a more extensive book of photos). The relative validity of the dietary method has been presented earlier (25). The energy-adjusted Pearson's validity correlation between the reference method (18 d of weighed food records during 1 yr) and the diet history method was for fat 0.64 and 0.69, for men and women, respectively. These values are higher than for comparable dietary methods in similar populations (26).

To calculate the relative (energy adjusted) intake of dietary fat, we regressed total fat intake (in grams) on total energy intake after logarithmic transformation (26). The residuals were saved and used to rank individuals and divide the study population into above and below median of the relative dietary fat intake. By using residuals of fat intake in relation to total energy intake instead of total fat intake to rank individuals, we reduced confounding from dietary over- and underreporting of energy. Because dietary patterns and self-reported dietary intake tend to differ according to gender (27, 28), fat intake above and below the median was calculated in male and female subjects separately. Furthermore, because a diagnosis of diabetes commonly leads to a change in dietary pattern, subjects with known diabetes (*i.e.* reporting a history of diabetes or being under treatment with antidiabetic agents) were excluded in the analyses.

Socioeconomic status was assessed on the basis of information on occupation, which was obtained from a self-administered socioeconomic and lifestyle questionnaire that was completed as part of a baseline examination as previously described (29). Occupational status was based on questions concerning job titles and actual work tasks, and individuals were classified into one of five categories: high-level nonmanual employees (*e.g.* business executives, engineers with a university degree, and university teachers); medium-level nonmanual employees (*e.g.* registered nurses, computer operators, and high school teachers); low-level nonmanual employees (*e.g.* office assistants, sales staff, and secretaries); skilled manual workers (*e.g.* vehicle mechanics, metal workers, and construction workers); and unskilled manual workers (*e.g.* factory workers, waiters, and cleaners). Based on these five categories, a variable containing two groups was created, with high- and medium-level nonmanual employees in one group and the other categories in the other group. Homemakers together with farmers and owners of business enterprises were excluded from the analyses because of their unclear status in relation to the other groups.

Alcohol consumption was divided into four categories. Individuals with no consumption of alcohol in the menu book and who indicated no consumption of alcohol during the previous year in the socioeconomic and lifestyle questionnaire were categorized as zero consumers. The other subjects were categorized into three groups according to their alcohol consumption (for women <15 g alcohol per day was considered as low, 15–30 g as medium, and >30 g high consumption; for men the corresponding figures were <20 g, 20–40 g, and >40 g).

Cigarette smoking was elicited by a self-administered questionnaire, with current cigarette smoking defined as any smoking within the past year.

The study protocols were approved by the Ethics Committee of Lund University. All participants provided written informed consent.

Statistical analyses

SPSS statistical software (version 17.0; Chicago, IL) was used for all analyses. Data were expressed as means ± SD. Because fasting concentrations of plasma TG, CRP, and insulin tended to be skewed to the right, all statistical analyses were performed after natural logarithmic transformation of these traits. We used sex-specific quartiles of copeptin, which were pooled in all analyses including both men and women because copeptin is known to be significantly higher in men. Frequency differences were evaluated using χ^2 test. Crude and multivariate adjusted logistic regression models were used to test the relationship between quartiles of copeptin and binary outcome variables, whereas crude and multivariate adjusted linear regression models were used to test the relationship between quartiles of copeptin and continuous outcome variables. A two-sided $P < 0.05$ was considered statistically significant.

Results and Discussion

Baseline population characteristics are shown in Table 1. The prevalence of MetS was on the average 21.5% and higher in men than in women. Level of copeptin (in quartiles, *i.e.* Q1–Q4) was positively associated with MetS, hypertension, waist, BMI, CRP, and TG and inversely associated with HDL after adjustment for age and sex (model 1 adjustment). This relationship remained for hypertension, waist, BMI, and CRP after further adjustment for insulin and DM (model 2 adjustment) (Table 2). For MetS variables that were significant after model 2 adjustments (Table 2), we additionally adjusted for Cystatin C. We found that hypertension (P trend = 0.02), CRP (P for trend <0.001), and waist (P for trend = 0.04) remained significantly associated with copeptin, whereas BMI (P for trend = 0.14) did not. In analyses adjusted for either glucose alone or glucose and insulin together in addition to age and sex in the nondiabetic population (n = 4377), we found that increasing copeptin remained positively associated to hypertension (P for trend = 0.003 and P for trend = 0.02, respectively), CRP (P for trend <0.001 and P for trend <0.001, respectively), and waist (P for trend <0.001 and P for trend = 0.03, respectively). BMI was associated with copeptin when adjusted for glucose, age, and sex but not when adjusted for glucose, age, sex, and insulin (P for trend <0.001 and P for trend = 0.12, respectively).

After model 2 adjustment the odds ratio (OR) for hypertension increased linearly in Q2-Q4 compared with the lowest quartile (Q1) of copeptin [1.04, 95% confidence interval (CI) 0.88–1.23; 1.07, 0.90–1.27, and 1.31, 1.10–

TABLE 1. Population description (n = 4742)

	Men+women	Men	Women
Age (yr)	57.5 ± 5.9[a]	57.7 ± 6.0	57.4 ± 5.9
Sex (percent men)	40.5	NA	NA
Copeptin (pmol/liter)[b]	5.14 (3.20–8.15)	7.07 (4.57–10.60)	4.23 (2.70–6.42)
Hypertension (%)	63.3	68.2	60.0
CRP (mg/liter)[b]	1.3 (0.7–2.8)	1.4 (0.7–2.8)	1.3 (0.7–2.8)
HDL (mmol/liter)	1.39 ± 0.37	1.21 ± 0.30	1.51 ± 0.37
TG (mmol/liter)[b]	1.14 (0.86–1.58)	1.27 (0.95–1.74)	1.07 (0.81–1.47)
Waist (cm)	83.5 ± 12.7	93.0 ± 9.9	77.0 ± 10.1
Abdominal obesity (%)	14.0	15.1	13.2
BMI (kg/m²)	25.7	26.1 ± 3.4	25.4 ± 4.1
Obesity (%)	12.5	11.4	13.2
Glucose (mmol/liter)	5.13 ± 1.27	5.34 ± 1.44	4.99 ± 1.12
Diabetes (%)	7.7	10.7	5.7
MetS	21.5	25.6	18.7
Insulin (mU/liter)[b]	6.0 (4.0–9.0)	7.0 (5.0–10.0)	6.0 (4.0–9.0)
Smoking (%)	25.6	26.5	25.1
Low socioeconomic status (%)	67.5	58.1	73.6
Alcohol (g/d)[b]	6.7 (1.5–14.4)	11.1 (3.6–21.8)	5.0 (0.8–10.5)
Fat intake (g/d)[b]	91 (71–115)	108 (87–134)	81 (65–100)
Low physical activity (%)	23.3	23.3	23.4

NA, Not applicable.

[a] Mean ± SD (all such values if nothing else specified).

[b] Expressed as median ± interquartile range.

1.57, respectively, in Q2–Q4, *P* for trend = 0.004]. Corresponding figures for abdominal obesity was 1.21, 0.92–1.60; 1.16, 0.88–1.54; 1.57, 1.20–2.05, respectively, *P* for trend = 0.002; for obesity, 1.25, 0.93–1.66; 1.15, 0.86–1.53; 1.49, 1.13–1.97, respectively, *P* for trend = 0.01; and for top quartile of CRP, 1.11, 0.91–1.36; 1.13, 0.93–1.38; 1.32, 1.09–1.61, *P* for trend = 0.007). Furthermore, in models that in addition to model 2 covariates included adjustment for all other MetS variables that were significantly related to copeptin (Table 2), copeptin remained significantly related to hypertension, abdominal obesity, and CRP and borderline significantly related to obesity (Fig. 1A–D). Finally, increasing quartile of copeptin was associated

with increased odds of MetS after model 1 adjustment (Fig. 1E), and the association between MetS and copeptin (in quartiles) remained in a model that additionally to age and sex was adjusted for BMI (OR 1.13, 95% CI 1.06–1.22, *P* for trend = 0.001), waist (OR 1.11, 95% CI 1.03–1.20, *P* trend = 0.005), or both of these measures of obesity simultaneously (OR 1.11, 95% CI 1.03–1.20, *P* trend = 0.005).

High levels of copeptin was associated with high fat intake and low physical activity and showed a borderline significant relationship with low socioeconomic status in linear regression models adjusted for age and sex and additionally adjusted for physical activity in analyses con-

TABLE 2. Components of the MetS in sex-specific quartiles of copeptin, which were pooled according to sex

	Q1 copeptin [0.01–4.59 in men, 0.01–2.71 in women (pmol/liter)]	Q2 copeptin [4.61–7.13 in men, 2.72–4.24 in women (pmol/liter)]	Q3 copeptin [7.14–10.6 in men, 4.25–6.44 in women (pmol/liter)]	Q4 copeptin [10.7–428 in men, 6.47–143 in women (pmol/liter)]	*P* crude	*P* model 1 adjusted[a]	*P* model 2 adjusted[b]
Hypertension	58.8%	61.5%	63.9%	69.4%	<0.001	<0.001	0.004
CRP[c]	1.1 (0.6–2.4)	1.3 (0.6–2.7)	1.4 (0.7–2.8)	1.6 (0.8–3.2)	<0.001	<0.001	<0.001
HDL	1.42 ± 0.37	1.39 ± 0.37	1.38 ± 0.38	1.37 ± 0.37	0.007	0.002	0.39
TG[c]	1.09 (0.82–1.46)	1.14 (0.85–1.58)	1.15 (0.87–1.63)	1.21 (0.90–1.64)	<0.001	<0.001	0.10
Waist	82.0 ± 12	83.4 ± 12	83.8 ± 13	84.9 ± 13	<0.001	<0.001	0.007
BMI	25.1 ± 3.6	25.8 ± 3.8	25.9 ± 3.7	26.1 ± 4.3	<0.001	<0.001	0.03
MetS	15.0%	21.5%	24.3%	25.5%	<0.001	<0.001	NA

P value in logistic or linear regression. NA, Not applicable.

[a] Model 1 is adjusted for age and sex.

[b] Model 2 is adjusted for age, sex, DM, and insulin.

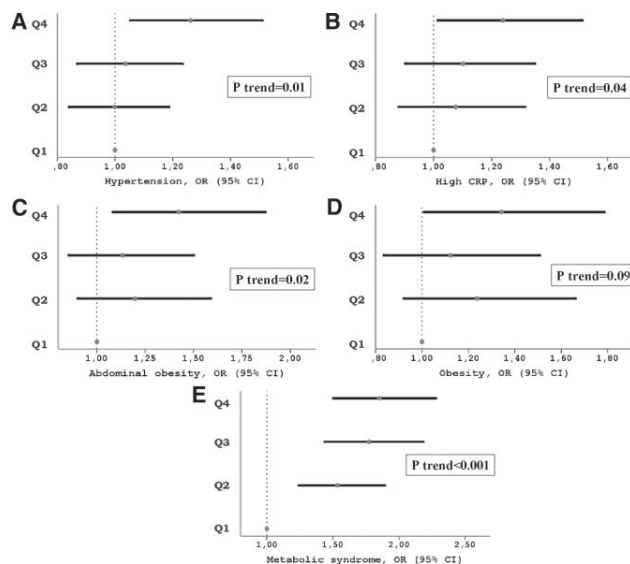[c] Expressed as median ± interquartile range.

FIG. 1. Hypertension, CRP, abdominal obesity, obesity, and MetS (according to Adult Treatment Panel III) in increasing quartile of copeptin (Q1–Q4) in models that in addition to age, sex, DM, and insulin (model 2) are adjusted for all other MetS variables that were significantly related to copeptin when adjusted for model 2 (Table 2). A, In addition to model 2, panel is adjusted for CRP, abdominal obesity, and obesity. B, In addition to model 2, panel is adjusted for hypertension, abdominal obesity, and obesity. C, In addition to model 2, panel is adjusted for hypertension and CRP. D, In addition to model 2, panel is adjusted for hypertension and CRP. E is adjusted for age and sex only.

association with physical activity remained significant only among men (Table 3).

In the next step, we investigated whether the association between quartiles of copeptin and components of the MetS was independent from the environmental factors fat intake, socioeconomic status and physical activity, which were all suggested to influence copeptin levels. We found that copeptin was associated with hypertension, CRP, waist, BMI, DM, hyperinsulinemia, and MetS independently of age, sex, fat intake, socioeconomic status, and physical activity (Table 4). Furthermore, because copeptin is known to be higher among men, we analyzed men and women separately to evaluate whether the association between copeptin quartiles and components of the MetS was independent of environmental factors in both men and women. These associations remained significant in men (Table 5) and significant or borderline significant in women (Table 6).

The causes of the clustering of cardiovascular risk factors within the MetS are still unknown, and reversal of the substantially increased cardiovascular risk associated with MetS requires identification of its basic pathophysiological factors. Elevated levels of circulating AVP, measured by copeptin, is not only linked to heart disease (30) and diabetic heart disease (31) but is also

cerning fat intake (Table 3). No significant associations were seen between levels of copeptin in plasma and smoking or alcohol consumption. When analyzing men and women separately, the association between copeptin and fat intake remained significant in both groups, whereas the

TABLE 3. Copeptin in relation to environmental factors

	All	P	Women	P	Men	P
High fat intake (known DM = 0)[a]	0.11 (0.05–0.17)	<0.001	0.12 (0.04–0.20)	0.003	0.09 (0.01–0.16)	0.02
Low socioeconomic status	0.05 [(−0.008) to 0.12]	0.09	0.04 [(−0.05) to 0.13]	0.41	0.07 [(−0.004) to 0.148]	0.06
Smoking	0.04 [(−0.03) to 0.10]	0.25	0.07 [(−0.02) to 0.16]	0.15	−0.005 [(−0.09) to 0.08]	0.90
Physical activity (Q1 vs. Q2–Q4)	0.09 (0.02–0.15)	0.01	0.07 [(−0.02) to 0.17]	0.13	0.11 (0.02–0.19)	0.02
Alcohol intake C1 and C2 vs. C3 and C4[b]	−0.005 [(−0.08) to 0.07]	0.90	−0.04 [(−0.16) to 0.08]	0.49	0.03 [(−0.05) to 0.11]	0.52
Alcohol habits C1 and C4 vs. C2 and C3[b]	0.03 [(−0.06) to 0.13]	0.51	−0.02 [(−0.16) to 0.13]	0.83	0.09 [(−0.03) to 0.21]	0.14

Values are given as β-coefficient (95% CI) expressed as number of SD increase of LN-transformed plasma copeptin concentration in relation to presence of the independent variable. All values are adjusted for age. Additionally, values are adjusted for sex in analyses with both men and women and adjusted for physical activity in analyses concerning fat intake.

[a] Dichotomous variable with above vs. below median of relative intake of dietary fat. We regressed total fat intake on total energy intake in males and females separately, and the residuals were saved and used to rank individuals.

[b] Alcohol consumption was divided into four categories (C1–C4) from lowest to highest consumption.

TABLE 4. Copeptin associated with components of the MetS in model adjusted for fat intake, socioeconomic status, and physical activity in men and women without known diabetes

	Increase per copeptin quartile	*P* trend
Hypertension	1.11 (1.05–1.18)	<0.001
Ln CRP[a]	0.07 (0.05–0.10)[a]	<0.001
Waist[a]	0.06 (0.04–0.08)[a]	<0.001
BMI[a]	0.07 (0.04–0.09)[a]	<0.001
DM	1.20 (1.06–1.36)	0.005
Hyperinsulinemia[b]	1.24 (1.16–1.32)	<0.001
MetS	1.16 (1.09–1.25)	<0.001

All analyses included age, copeptin quartiles, physical activity, socioeconomic status, and fat intake as covariates. Data are expressed as OR (95% CI) if nothing else specified.

[a] Expressed as *β*-coefficient (95% CI) of z-score of continuous variable.

[b] In subjects without DM at baseline, using Q copeptin-specific for subjects without DM at baseline.

TABLE 6. Copeptin associated with components of the MetS in model adjusted for fat intake, socioeconomic status, and physical activity in women without known diabetes

	Increase per copeptin quartile	*P* trend
Hypertension	1.08 (1.00–1.16)	0.054
Ln CRP[a]	0.06 (0.02–0.09)[a]	0.001
Waist[a]	0.05 (0.03–0.08)[a]	<0.001
BMI[a]	0.07 (0.03–0.10)[a]	<0.001
DM	1.20 (1.00–1.44)	0.053
Hyperinsulinemia[b]	1.21 (1.11–1.32)	<0.001
MetS	1.13 (1.03–1.24)	0.009

All analyses included age, copeptin quartiles, and physical activity, socioeconomic status, and fat intake as covariates. Data are expressed as OR (95% CI) if nothing else specified.

[a] Expressed as *β*-coefficient (95% CI) of z-score of continuous variable.

[b] In women without DM at baseline, using Q copeptin specific for women without DM at baseline.

a powerful predictor of future development of DM (10), suggesting that elevated AVP has a central role for development of DM-associated cardiovascular risk factors, such as MetS. Interestingly, Saleem *et al.* (11) recently found a cross-sectional association between plasma copeptin and MetS and components of the MetS in a population with familial hypertension and a relatively high prevalence of MetS (∼50%). Here we extend these findings in a large population-based sample by showing that high copeptin not only is associated with the clustering of the MetS components but also displays independent relationships with core components of the syndrome, *i.e.* hypertension and abdominal obesity, as well as with low-grade inflammation. The fact that copeptin was significantly related to these three potent cardiometabolic risk factors in a graded manner, even after adjustment for

TABLE 5. Copeptin associated with components of the MetS in model adjusted for fat intake, socioeconomic status and physical activity in men without known diabetes

	Increase per copeptin quartile	*P* trend
Hypertension	1.17 (1.06–1.29)	0.001
Ln CRP[a]	0.09 (0.05–0.14)[a]	<0.001
Waist[a]	0.07 (0.04–0.10)[a]	<0.001
BMI[a]	0.07 (0.03–0.10)[a]	<0.001
DM	1.20 (1.01–1.42)	0.04
Hyperinsulinemia[b]	1.27 (1.15–1.41)	<0.001
MetS	1.21 (1.09–1.34)	<0.001

All analyses included age, copeptin quartiles, and physical activity, socioeconomic status, and fat intake as covariates. Data are expressed as OR (95% CI) if nothing else specified.

[a] Expressed as *β*-coefficient (95% CI) of z-score of continuous variable.

[b] In men without DM at baseline, using Q copeptin specific for men without DM at baseline.

each other as well as for DM and insulin resistance (Fig. 1, A–D) that we previously showed to be strongly independently related to plasma copeptin (10) suggests that the AVP system may be a unifying link with potential causal relationship to MetS. At the same time, we do acknowledge that the greatest limitation of our study is its cross-sectional design, which makes it impossible to draw conclusions about causality. Thus, our study makes prospective studies relating plasma copeptin level to future development of MetS and its core components warranted.

From a therapeutic point of view, should future studies indicate causality, the AVP system is an attractive target for MetS interventions because it is pharmacologically modifiable (32, 33). In addition, we pointed out several environmental factors implied in MetS, which we found to be associated with elevated copeptin levels, such as low physical activity, high fat intake, and low socioeconomic status. However, adjustment for these environmental factors did not affect the associations between copeptin and MetS or components of the MetS. This argues against a major role of low physical activity, high fat intake, and low socioeconomic status in explaining the copeptin-MetS relationship; however, we cannot exclude such a role due to the rough measurements of these factors as well as the cross-sectional design of our study.

Several reports have shown that stress is associated with MetS (34). Acute and chronic stressful stimuli activate the hypothalamic-pituitary-adrenal axis. AVP and CRH are the main regulators of the hypothalamic-pituitary-adrenal system and induces ACTH release. AVP and CRH release is situation specific, and the level of AVP is reported to be higher than CRH after certain stressful stimuli (35). Furthermore, the AVP-induced ACTH release has been reported to be resistant to glucocorticoid

feedback (36), suggesting that neuroendocrine stress elevates AVP levels thereby increasing the risk of developing MetS.

Another possible explanation to our findings is a genetic predisposition to elevated copeptin levels and metabolic syndrome in certain individuals. Knockout mice that lack expression of the V1aR have elevated AVP levels, insulin resistance, fat-induced DM, and obesity as well as altered fat metabolism when compared with wild-type mice (3, 37), suggesting that V1aR impairment is followed by a compensatory rise in AVP levels and development of components of the MetS. It is unknown how reduced V1aR signaling and high AVP levels in the V1aR knockout mouse leads to metabolic abnormalities; however, one potential explanation is that its elevated AVP levels overstimulate the V1bR because mice lacking the V1bR get improved glucose tolerance and insulin sensitivity.

In conclusion, our data suggest that altered activity of the AVP system may be a unifying factor in the pathogenesis of MetS and point to a new pharmacologically modifiable system of potential importance in the treatment of MetS and its consequences.

Acknowledgments

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IV

Open

ORIGINAL ARTICLE

Copeptin, a marker of vasopressin, in abdominal obesity, diabetes and microalbuminuria: the prospective Malmö Diet and Cancer Study cardiovascular cohort

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BACKGROUND: High plasma copeptin (copeptin), the C-terminal fragment of arginine vasopressin pro-hormone, has been associated with the metabolic syndrome (MetS), diabetes mellitus (DM) development and nephropathy. Here we tested whether elevated copeptin level is associated with later development of the MetS, its individual components and microalbuminuria.
METHODS: We analysed copeptin at baseline (1991–1994) in the population-based Malmö Diet and Cancer Study cardiovasular cohort and re-examined 2064 subjects 15.8 years later (mean age 72.8 years, 59% women) with oral glucose tolerance test and measurement of MetS and its individual components.
RESULTS: After age and sex adjustment, increasing quartiles of copeptin at baseline (the lowest quartile as reference) were associated with MetS (P for trend = 0.008), incident abdominal obesity (P for trend = 0.002), DM (P for trend = 0.001) and microalbuminuria (P for trend = 0.002). After additional adjustment for all the MetS components at baseline, increasing copeptin quartiles predicted incident abdominal obesity (odds ratios 1.55, 1.30 and 1.59; P for trend = 0.04), DM (odds ratios 1.18, 1.32 and 1.46; P for trend = 0.04) and microalbuminuria (odds ratios 1.05, 1.08 and 1.65; P for trend = 0.02) but not MetS (P for trend = 0.19) at the reexamination. Further, the relationship between copeptin and microalbuminuria was independent of baseline C-reactive protein, incident DM and incident hypertension.
CONCLUSION: Copeptin independently predicts DM and abdominal obesity but not the cluster of MetS. Apart from predicting DM and abdominal obesity, elevated copeptin signals increased risk of microalbuminuria. Interestingly, the association between copeptin and later microalbuminuria was independent of both prevalent and incident DM and hypertension. Our findings suggest a relationship between a dysregulated vasopressin system and cardiometabolic risk, which could have implications for risk assessment and novel preventive treatments.

International Journal of Obesity advance online publication, 22 May 2012; doi:10.1038/ijo.2012.88

Keywords: copeptin; arginine vasopressin; abdominal obesity; diabetes; metabolic syndrome; microalbuminuria

INTRODUCTION

Arginine vasopressin (AVP), which is also called antidiuretic hormone, is released from the neurohypophysis as a response to increased plasma osmolality and decreased blood volume. AVP exerts an antidiuretic effect in the kidney and a vasoconstrictive and blood platelet-aggregating effect in the vessels. In addition, animal studies have shown effects of AVP on glucose metabolism. AVP influences gluconeogenesis and glycogenolysis in the liver,[1,2] insulin and glucagon release by the Langerhans islets of the pancreas[3] and adrenocorticotrophic hormone release from the anterior hypophysis.[4] Vasopressin is a short-lived peptide and most assays have relatively limited sensitivity. An assay has been developed to measure plasma copeptin (copeptin), the C-terminal portion of the precursor of AVP. Copeptin is considered to be a reliable and clinically useful surrogate marker for AVP.[5]

In a Swedish population-based sample, we recently found elevated copeptin to be associated with incident diabetes mellitus (DM) independently of all clinically used DM confounders including plasma glucose and insulin.[6] Further, in the same population,

we found elevated copeptin to be cross-sectionally associated with the metabolic syndrome (MetS), obesity, hypertension, abdominal obesity and high C-reactive protein (CRP), suggesting that the AVP system is an underlying factor behind the MetS. However, we did not find any association between copeptin and triglycerides (TGs) or high-density lipoprotein (HDL) levels.[7]

Microalbuminuria, a common and early sign of target-organ damage in DM, hypertension and MetS, is associated with vascular dysfunction and is an independent potent risk factor for cardiovascular disease.[8–10] To improve cardiovascular prognosis and reduce morbidity and mortality in individuals prone to DM development and in patients with established DM, it is thus important to identify mechanisms leading to microalbuminuria and other signs of target-organ damage. Previous studies in humans and animals suggest a role for AVP in diabetic nephropathy, microalbuminuria and renal failure,[11–15] and recent studies show that elevated copeptin is associated with renal function decline in transplant recipients[16] and cross-sectionally associated with microalbuminuria in humans.[17] Further, copeptin

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is associated with cardiovascular events among patients with end-stage renal disease and type 2 DM.[18]

In the current study, we now aim to expand our previous findings and evaluate if elevated copeptin is related not only cross-sectionally to MetS, obesity, hypertension and abdominal obesity[7] but also longitudinally to future development of these conditions. Further, we aim to validate our previous finding that copeptin is associated to future development of DM by sharpening the DM end point, previously ascertained through registers, by a reexamination including an oral glucose tolerance test (OGTT). Finally, to further explore the possible involvement of AVP in target-organ damage, we test whether copeptin predicts development of microalbuminuria.

MATERIALS AND METHODS

Subjects
The Malmö Diet and Cancer Study (MDC) is a population-based prospective cohort consisting of 28 449 persons surveyed in 1991–1996.[19] From this cohort, 6103 persons were randomly selected to be studied for the epidemiology of carotid artery disease, and this sample is referred to as the MDC cardiovascular cohort. Fasting plasma samples were obtained in 5405 subjects in the MDC cardiovascular cohort.[20] Of those, complete data on covariates, including components of the MetS, potential confounders and copeptin, were available in 5131 individuals.

Baseline examination. Baseline measurements in plasma and whole blood were performed in overnight fasting samples. Analyses of fasting plasma lipids and whole-blood glucose were carried out at the time of baseline and follow-up examination at the Department of Clinical Chemistry, Skane University Hospital in Malmö, which is attached to a national standardization and quality control system. CRP was measured by a high-sensitivity assay (Tina-quant CRP; Roche Diagnostics, Basel, Switzerland). Cystatin C was measured using a particle-enhanced immuno-nephelometric assay (N Latex Cystatin C; Dade Behring, Deerfield, IL, USA). Copeptin was measured in fasting plasma samples using a commercially available assay in the chemiluminescence/coated tube format (B.R.A.H.M.S AG, Hennigsdorf, Germany) as described previously.[21] Mid-regional atrial natriuretic peptide was measured using an immunoluminometric sandwich assay targeted against amino acids in the mid-region of the peptide (B.R.A.H.M.S AG).[22] The motif for measuring cystatin C is that cystatin C is a good marker of glomerular filtration rate. Adjusting for cystatin C may thus be important in order to minimize bias related to variation of glomerular filtration rate as copeptin is largely cleared by glomerular filtration.

According to the National Cholesterol Education Program-Adult Treatment Panel III (NCEP ATP-III) criteria,[23] we classified subjects as having the MetS if they had three or more of the following characteristics: waist circumference >102 cm in men and >88 cm in women; fasting whole-blood glucose $\geqslant 5.4$ mmol l^{-1} (corresponding to fasting plasma glucose concentration of $\geqslant 6.1$ mmol l^{-1}) or treatment for diabetes; HDL <1.0 mmol l^{-1} in men and <1.3 mmol l^{-1} in women; TGs $\geqslant 1.7$ mmol l^{-1}; and systolic blood pressure (BP) $\geqslant 130$ mm Hg and/or diastolic BP $\geqslant 85$ mm Hg or use of anti-hypertensive medication (AHT). DM at baseline was defined as self-report of a physician diagnosis or use of diabetes medication or fasting whole blood glucose $\geqslant 6.1$ mmol l^{-1} (corresponding to fasting plasma glucose concentration of $\geqslant 7.0$ mmol l^{-1}). Abdominal obesity was defined as waist circumference >102 cm in men and >88 cm in women. Obesity was defined as body mass index $\geqslant 30$ kg m^{-2}. BP was measured using a mercury-column sphygmomanometer after 10 min of rest in the supine position. Hypertension at baseline was defined as baseline systolic BP $\geqslant 140$ mm Hg, diastolic BP $\geqslant 90$ mm Hg or use of AHT according to a baseline questionnaire or a 7-day menu book.

Reinvestigation and end points. Of the 5131 individuals at baseline, 2064 individuals had been reinvestigated from January 2007 to March 2010 (participation rate 70% of those invited), resulting in a mean follow-up time of 15.8 years. At the reinvestigation, we used a protocol similar to that applied at the baseline examination but with additional measurement of urine albumin excretion and an OGTT (75 g glucose) after an overnight fast, with measurement of plasma glucose at time 0 (before) and 120 min after glucose ingestion.

In the analyses of incidence of MetS, abdominal obesity, obesity, hypertension and DM, we excluded subjects with baseline prevalence of the corresponding outcome variable (Tables 1 and 2).

Table 1. Population description—baseline characteristics ($n = 2064$)

Age (years)	57.0 ± 5.7
Sex (% men)	40.9
Waist circumference (cm)	83.0 ± 12.6
Abdominal obesity	265 (12.8)
BMI (kg m^{-2})	25.6 ± 3.7
Obesity	232 (11.2)
HDL (mmol l^{-1})	1.39 ± 0.37
Triglycerides (mmol l^{-1})[a]	1.14 (0.84–1.59)
Glucose (mmol l^{-1})[a]	4.9 (4.6–5.2)
Diabetes mellitus	136 (6.6)
Systolic BP (mm Hg)	140 ± 18
Diastolic BP (mm Hg)	86 ± 9.1
Hypertension	1263 (61.2)
AHT	333 (16.1)
Metabolic syndrome	411 (19.9)
Cystatin C (mg l^{-1})	0.77 ± 0.13
CRP (mg l^{-1})[a]	1.2 (0.6–2.5)
Copeptin (pmol l^{-1})[a]	5.08 (3.19–8.09)

Abbreviations: AHT, anti-hypertensive treatment; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; HDL, high-density lipoprotein. Values are presented as mean $\pm$ s.d. or n (%) (if not otherwise specified). [a]Expressed as median (interquartile range).

DM at reinvestigation was defined as self-report of a physician diagnosis or use of DM medication after the baseline examination, or fasting plasma glucose of $\geqslant 7.0$ mmol l^{-1} or 120-min value post OGTT plasma glucose >11.0 mmol l^{-1}.

The prevalence of hypertension at baseline was as high as 61.2% (Table 1). Instead of excluding 61.2% of the population in the incidence analysis of hypertension, we only excluded subjects on AHT at baseline and used initiation of AHT during follow-up as the end point, assuming that initiation of AHT is a more valid indicator of a diagnosis of hypertension. However, as a secondary analysis, we excluded all the subjects with prevalent hypertension at baseline and used the broader definition in the incidence analysis of hypertension (systolic BP $\geqslant 140$ mm Hg, diastolic BP $\geqslant 90$ mm Hg or use of AHT at the reinvestigation). Albumin and creatinine were measured in morning urine samples with methods described earlier[24] and microalbuminuria at reinvestigation was defined according to the Swedish upper (95%) reference limits of $\geqslant 3.0$ g albumin per mol creatinine in a morning urine sample.[24] The definitions of abdominal obesity, obesity and MetS at reinvestigation were the same as those at the baseline examination.

Statistics
SPSS statistical software (version 17.0, SPSS Inc., Chicago, IL, USA) was used for all analyses. Skewed variables were logarithmically transformed before analysis. We used sex-specific quartiles of copeptin, which were pooled according to sex in all the analyses, as copeptin is known to be significantly higher in men. Multivariate-adjusted logistic and linear regression models were used to test the relationship between quartiles of copeptin and the different outcome variables. A two-sided P-value <0.05 was considered statistically significant.

RESULTS
The average follow-up time was 15.8 years. During the follow-up, 433 subjects (26.2%) developed MetS, 236 (12.9%) developed obesity, 533 (29.6%) developed abdominal obesity, 730 (42.2%) started AHT treatment, 458 (57.2%) developed hypertension and 308 (16.0%) developed new-onset DM. The prevalence of microalbuminuria at baseline investigation was not measured, but the prevalence at reinvestigation was 191 (9.3%) among subjects with DM at baseline and 159 (8.2%) among subjects without DM at baseline. Medians (interquartile range) of copeptin (in pmol l^{-1}) in quartiles 1–4 were 3.16 (2.21–3.80), 5.56 (4.86–6.27), 8.44 (7.64–9.53) and 13.5 (11.50–16.60), respectively, in men, and 1.86 (1.36–2.34), 3.41 (3.04–3.77), 5.14 (4.70–5.76) and 8.41 (7.22–10.45), respectively, in women.

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Table 2. Baseline plasma copeptin quartiles (Q1–Q4) in relation to incidence of components of the metabolic syndrome and microalbuminuria at reinvestigation after 15.8 years of follow-up

	Model 1		Model 2	
	OR (95% CI)[a]	P trend	OR (95% CI)[b]	P trend
Abdominal obesity, subjects without abdominal obesity at baseline (n = 1799)				
Q1	1.00 (ref)	0.003	1.00 (ref)	0.04
Q2	1.30 (0.97–1.74)		1.55 (1.08–2.21)	
Q3	1.37 (1.02–1.83)		1.30 (0.91–1.84)	
Q4	1.58 (1.18–2.12)		1.59 (1.11–2.28)	
Obesity, subjects without obesity at baseline (n = 1832)				
Q1	1.00 (ref)	0.21	1.00 (ref)	0.86
Q2	1.26 (0.85–1.87)		1.17 (0.76–1.80)	
Q3	1.24 (0.83–1.83)		1.13 (0.74–1.73)	
Q4	1.31 (0.88–1.95)		1.06 (0.69–1.63)	
Incident AHT, subjects without AHT at baseline (n = 1731)				
Q1	1.00 (ref)	0.62	1.00 (ref)	0.45
Q2	1.11 (0.84–1.46)		1.06 (0.79–1.43)	
Q3	1.15 (0.88–1.51)		1.00 (0.74–1.34)	
Q4	1.06 (0.80–1.39)		0.90 (0.66–1.22)	
Diabetes mellitus, subjects without diabetes at baseline (n = 1928)				
Q1	1.00 (ref)	0.001	1.00 (ref)	0.04
Q2	1.42 (0.98–2.06)		1.18 (0.79–1.76)	
Q3	1.49 (1.03–2.15)		1.32 (0.89–1.96)	
Q4	1.87 (1.30–2.67)		1.46 (0.99–2.16)	
Metabolic syndrome, subjects without metabolic syndrome at baseline (n = 1653)				
Q1	1.00 (ref)	0.008	1.00 (ref)	0.19
Q2	1.25 (0.91–1.72)		1.21 (0.85–1.72)	
Q3	1.23 (0.90–1.68)		1.05 (0.74–1.49)	
Q4	1.57 (1.15–2.14)		1.34 (0.95–1.91)	
Microalbuminuria, subjects with and without diabetes at baseline (n = 2064)				
Q1	1.00 (ref)	0.002	1.00 (ref)	0.02
Q2	1.19 (0.74–1.91)		1.05 (0.65–1.69)	
Q3	1.25 (0.79–2.00)		1.08 (0.67–1.73)	
Q4	1.97 (1.27–3.04)		1.65 (1.06–2.59)	
Microalbuminuria, subjects without diabetes at baseline (n = 1928)				
Q1	1.00 (ref)	0.005	1.00 (ref)	0.02
Q2	1.26 (0.76–2.09)		1.14 (0.69–1.91)	
Q3	1.14 (0.68–1.90)		1.01 (0.60–1.70)	
Q4	2.01 (1.26–3.22)		1.78 (1.11–2.88)	

Abbreviations: AHT, anti-hypertensive treatment; CI, confidence interval; OR, odds ratio. Subjects with baseline prevalence of the outcome variable in question (shown in Table 1) were excluded from the analyses. Medians (interquartile range) of copeptin (in pmol l^{-1}) in quartiles 1–4 were 3.16 (2.21–3.80), 5.56 (4.86–6.27), 8.44 (7.64–9.53) and 13.5 (11.50–16.60), respectively, in men and 1.86 (1.36–2.34), 3.41 (3.04–3.77), 5.14 (4.70–5.76) and 8.41 (7.22–10.45), respectively, in women. [a]Adjusted for follow-up time, age and sex. [b]Adjusted for follow-up time, age, sex, cystatin C, hypertension (systolic blood pressure and diastolic blood pressure in analyses with incident AHT as outcome), glucose, triglycerides, high-density lipoprotein and waist circumference.

Increasing quartiles of baseline copeptin predicted incident MetS in a model adjusted for baseline age, sex and follow-up time (model 1), but the association did not remain significant when adjusted further for baseline hypertension, glucose, TGs, HDL, waist, cystatin C and follow-up time (model 2) (Table 2, Figure 1).

We then analysed the longitudinal relationship between baseline copeptin and incident abdominal obesity, DM and incident AHT, respectively, as these individual MetS components were

previously found to be cross-sectionally associated with elevated copeptin.[7] Increasing quartile of copeptin predicted both incident abdominal obesity and incident DM when adjusted for model 1 covariates, as well as after full adjustment for model 2 covariates (baseline age, sex, hypertension, glucose, TG, HDL, waist, cystatin C and follow-up time) (Table 2, Figure 1). When the prospective relationship between baseline copeptin and incident DM was additionally adjusted for incident abdominal obesity on top of model 2 covariates, it remained significant (odds ratio (95% confidence interval (CI)) in quartiles 1–4: 1.00 (reference), 1.17 (0.78–1.74), 1.31 (0.88–1.94) and 1.45 (0.98–2.14), respectively; P for trend = 0.049). Similarly, we adjusted the relationship between baseline copeptin and incident abdominal obesity for incident DM on top of model 2 covariates, and this relationship remained significant also (odds ratio (95% CI): 1.00 (reference), 1.55 (1.08–2.21), 1.30 (0.91–1.85) and 1.60 (1.12–2.29), respectively; P for trend = 0.03). We did not find any longitudinal relationship between baseline copeptin and incident AHT (Table 2). Neither was there any significant relationship between baseline copeptin and incident hypertension (P = 0.65). Copeptin was previously found to be borderline significantly and cross-sectionally associated with obesity,[7] but we did not find any longitudinal relationship between baseline copeptin and incident obesity (Table 2).

Increasing quartiles of copeptin was associated with ΔHDL, ΔLDL and ΔTG (all expressed as mM change per year). In a model adjusted for age and sex, increasing quartiles of copeptin was associated with ΔHDL (beta coefficient (95% CI) −0.001 (−0.002 to −0.0002); P = 0.007) and ΔLDL (−0.005 (−0.008 to −0.002); P = 0.002), whereas there was no association with ΔTG (−0.0002 (−0.002 to 0.001); P = 0.75). When age, sex, cystatin C, hypertension, glucose, TG, HDL and waist circumference (and LDL in analysis with ΔLDL as outcome) were added to the model, only ΔHDL remained negatively associated with increasing copeptin quartile (−0.001 (−0.002 to −0.0003); P = 0.002), whereas ΔLDL and ΔTG did not (−0.001 (−0.004 to 0.001); P = 0.28 and 0.001 (−0.001 to 0.002); P = 0.39, respectively).

Increasing quartiles of copeptin at baseline was associated with microalbuminuria at reinvestigation after model 1 adjustments, as well as after model 2 adjustments, both before and after exclusion of prevalent DM from the cohort (Table 2, Figure 1). As there is a strong link between CRP and microalbuminuria,[25] we further adjusted for baseline CRP on top of model 2 and found that the association across increasing copeptin quartiles among subjects free from diabetes at baseline was not affected (odds ratio (95% CI): 1.0 (reference), 1.14 (0.68–1.91), 1.03 (0.61–1.73) and 1.91 (1.18–3.09); P for trend = 0.01). Finally, on top of model 2 and CRP, we additionally adjusted for incident hypertension and DM and found that the association across increasing quartiles of copeptin remained significant (odds ratio (95% CI): 1.00 (reference), 1.09 (0.65–1.83), 0.97 (0.57–1.66) and 1.82 (1.12–2.96); P for trend = 0.02).

As we recently reported that mid-regional atrial natriuretic peptide predicts the development of DM in our study population,[26] we additionally adjusted for mid-regional atrial natriuretic peptide in analyses, relating copeptin to incidence of DM, abdominal obesity and microalbuminuria. When adding mid-regional atrial natriuretic peptide as a covariate on top of model 2 (Table 2), copeptin remained significantly associated with DM (P = 0.04), abdominal obesity (P = 0.04) and microalbuminuria (P = 0.02) (Supplementary Table S1).

DISCUSSION
The key finding of this study is that an elevated copeptin at baseline predicts incident DM, abdominal obesity and microalbuminuria during a long-term follow-up (15.8 years on average). These results extend our previous cross-sectional findings that
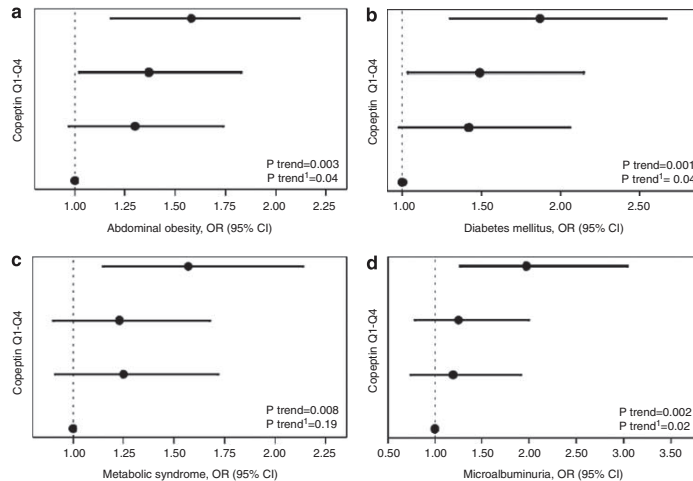
Figure 1. Baseline plasma copeptin quartiles (Q1–Q4) in relation to incidence of components of the MetS (**a**–**c**) and microalbuminuria (**d**) at reinvestigation in model adjusted for follow-up time, age and sex. Model adjusted for follow-up time, age, sex, cystatin C, hypertension, glucose, TGs, HDL and waist circumference.

copeptin is associated with abdominal obesity,[7] by showing that this association is also valid prospectively. Furthermore, in the current results, incident DM was based on a reinvestigation including an OGTT. Thus, they extend and strengthen our previous finding that copeptin independently predicted DM where the DM end point was based only on register data.[6]

Copeptin and MetS

Interestingly, despite of the prospective relationship between copeptin and two core components of the MetS (abdominal obesity and DM) and our previously observed cross-sectional association between copeptin and MetS,[7] in the current study, copeptin was not an independent predictor of the cluster of MetS. Our previous finding of cross-sectional relationship between copeptin and MetS was probably driven by the association between copeptin and both DM and abdominal obesity, respectively.

Copeptin, abdominal obesity and DM

The fact that copeptin was elevated many years before the development of overt DM and abdominal obesity, and remained significantly associated independently of a broad range of potential confounders, suggests a primary role for the AVP system in the pathophysiology of DM and abdominal obesity. Assuming that there is such a primary role of AVP, one wonders whether AVP induces development of abdominal obesity and DM independently of each other or whether development of the two closely related end points is dependent upon each other. As the association between baseline copeptin and incident DM was independent of incident abdominal obesity and vice versa, it is possible that AVP triggers two different pathways leading to DM and abdominal obesity, independently. However, as we do not have repeated measurements of waist circumference and glycaemia during the follow-up period, it is possible that we have missed temporal trends occurring during that time frame. Thus, we cannot exclude the possibility that a primary elevation of AVP leads to DM development by increasing abdominal fat depositions.

Even though metabolic effects of AVP may be expected, it is not yet elucidated as to how AVP favour obesity and DM. AVP mediates gluconeogenesis and glycogenolysis through vasopressin 1a receptors (V1aRs) in the liver[1,2] and stimulates the secretion of either glucagon or insulin, depending on the actual level of glycaemia, through vasopressin 1b receptors (V1bRs) in pancreatic islets.[3] Further, AVP exerts an anti-lipolytic action, possibly through haemodynamic effects.[27] However, the contribution of AVP to glucose and lipid metabolism seems to be rather complex. Mice with selective deletion of V1aR exhibit elevated glucose levels, predisposition for obesity and diabetes, low TG levels and enhanced lipid metabolism,[28,29] whereas mice lacking V1bR display a phenotype of low glucose levels and better insulin sensitivity.[30] In humans, the rs1042615 polymorphism of the V1aR gene was among men associated with altered body mass index before and after walking training and features resembling the phenotype of the mouse with V1aR deletion, including elevated glucose levels and low TG levels, as well as increased DM prevalence in subjects with a high-fat intake or overweight.[31,32]

Moreover, AVP binding to V1bR in the anterior hypophysis mediates adrenocorticotrophic hormone release and elevate glucocorticoid levels in plasma. Indeed, mice lacking the V1bR show lower levels of corticosterone in plasma both under stress and during basal conditions.[33] The AVP-induced adrenocorticotrophic hormone release has been reported to be resistant to glucocorticoid feedback in contrast to the corticotropin-releasing hormone-induced adrenocorticotrophic hormone release.[34]

The proportion of subjects who developed new-onset DM was substantially higher in this study than in our previous study.[6] This difference in DM incidence was expected as the DM end point in our previous study was register-based, requiring subjects to have been in contact with the health-care system in order to be captured, whereas in the current study, we screened all the subjects for DM with OGTT and fasting plasma glucose measurements. In addition, the follow-up time was longer in the current than in our previous study. In any case, whichever method used to retrieve incident cases of DM, copeptin was an independent predictor of DM.

Copeptin and microalbumiuria

Apart from constituting the core of the diagnosis of diabetic nephropathy in DM, microalbuminuria is considered as an early and validated sign of organ damage of the cardiovascular system not only in patients with hypertension and DM but also in the general population.[8–10,35] In the current study, the proportion of subjects with microalbuminuria at reexamination in the present study (9.3% and 8.2% among subjects who had or did not have DM at baseline, respectively) appears to be higher than that seen in other Caucasian populations.[36] This may be explained by the relatively high mean age at the reinvestigation (72.8 ± 5.6 years); in fact, few studies have examined the prevalence of micro-albuminuria in general populations with a mean age > 70 years. Further, the prevalence of microalbuminuria in hypertensive individuals ranges 7–40% (depending on age, race, and ethnicity) and the prevalence of microalbuminuria in patients with DM ranges 30–40%.[9]

Our finding that copeptin level at baseline is associated with microalbuminuria after long-term follow-up independent of baseline MetS variables and CRP supports previous cross-sectional findings in humans[11,17] and suggests a role for the AVP system in the development of microalbuminuria. The association between copeptin and microalbuminura could be speculated to be explained by AVP-mediated changes of glucose metabolism or BP during follow-up. However, the association was present whether or not subjects with DM at baseline were included, and it remained significant after adjustment for all MetS factors, renal function (as assessed with cystatin C) and CRP at baseline, as well as for both incident DM and hypertension. This suggests that microalbuminuria may be at least partly directly dependent upon AVP and not mediated by other cardiometabolic risk factors related to copeptin such as DM and abdominal obesity.

Regarding the pathway whereby AVP may increase albuminuria, there are some data suggesting that AVP might contribute to rise in albumin excretion as a consequence of its antidiuretic effect mediated by vasopressin 2 receptors (V2Rs).[11,13,14] Further, V2R may have a role in renal function decline. AVP suppression lowered proteinuria and improved renal function in rats with subtotal nephrectomy.[12,37] Conversely, chronic infusion of dDAVP, a V2R agonist, exaggerated proteinuria in a rat model of renal failure.[15] Thus, in contrast to the relationship between AVP, DM and abdominal obesity, which is most likely dependent upon V1aR and/or V1bR, experimental evidence point at the V2R as a link between AVP and microalbuminuria.

Strengths and weaknesses of the study

Our study is large, prospective and has a long follow-up time. All our analyses included adjustment for a broad range of potential confounding factors, most importantly baseline levels of all factors of the MetS, cystatin C (to account for differences in glomerular filtration rate) and, in extended analyses, even incident DM, abdominal obesity and hypertension. The independent prospective relationship between copeptin at baseline and DM, abdominal obesity and microalbuminuria suggests a primary role of the AVP system as a cardiometabolic risk factor and warrants further studies testing whether interventions targeted at the AVP system may in fact prevent or reverse glucose intolerance, abdominal obesity and microalbuminuria. However, we do acknowledge limitations. First, microalbuminuria was not measured at baseline, preventing any firm statement about progression from normo- to microalbuminuria. Second, it was not possible to prospectively analyse copeptin in relation to CRP and insulin, variables that were previously found to be cross-sectionally associated with elevated copeptin,[6,7] as these variables were not measured at reinvestigation. Third, subjects who participated in the MDC cardiovascular cohort baseline exam but died during follow-up or did not participate in the reinvestigation for

other reasons are missing. This could lead to either over- or underestimation of the strength of the prospective relationship between copeptin at baseline and the studied end points. However, comparison of copeptin levels between participants and non-participants of the reinvestigation did not reveal any significant difference ($P = 0.46$), suggesting that such potential bias is of limited magnitude.

CONCLUSIONS

In this large prospective population-based cohort, copeptin predicts abdominal obesity, DM and microalbuminuria, suggesting a primary role for the AVP system in development of these conditions. These findings may have implications for risk assessment and warrant further studies testing whether interventions targeted at the AVP system may prevent the development of DM and reduce cardiometabolic risk.

CONFLICT OF INTEREST

Drs Struck and Morgenthaler are employees of B.R.A.H.M.S GmbH, which holds patent rights on the copeptin assay. The remaining authors declare no conflict of interest.

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Supplementary Information accompanies the paper on International Journal of Obesity website (http://www.nature.com/ijo)

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Genetic vasopressin 1b Receptor variance in overweight and diabetes mellitus

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Running title: AVP receptor 1b in overweight

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Abstract

Objective: Recently, imbalance in the vasopressin (AVP) system, measured as elevated levels of copeptin (the c-terminal part of the AVP pro-hormone) in plasma, was linked to development of abdominal obesity and diabetes mellitus (DM). Here, we aim to investigate if genetic variation of the human AVP receptor 1b gene (AVPR1B) is associated with measures of obesity and DM.

Design: Malmö Diet and Cancer study (MDC) is a population-based prospective cohort examined 1991-1996. Four tag SNPs (rs35810727, rs28373064, rs35439639, rs35608965) of AVPR1B were genotyped in the cardiovascular cohort (n=6 103) of MDC (MDC-CC) and associated with measures of obesity and DM. Significant SNPs were replicated in another 24 344 MDC individuals (MDC replication cohort).

Results: In MDC-CC, the major allele of rs35810727 was associated with elevated BMI (beta-coefficient ± SE; 0.30 ± 0.14, P=0.03) and waist (0.78 ± 0.36, P=0.03) after age and gender adjustment. The association with BMI was replicated in the MDC replication cohort (0.21 ± 0.07, P=0.003), whereas that with waist was not significant. In MDC-CC there was no association between the major allele of rs35810727 and DM, but in the complete MDC cohort (n=30 447) the major allele of rs35810727 was associated with DM (OR (95% CI); 1.10 (1.00-1.20), P=0.04).

Conclusions: Genetic variance of AVPR1B contributes to overweight. Furthermore, our data indicate a link between AVPR1B variance and DM development. Our data point at a causal relationship between disturbance of the pharmacologically modifiable AVP system and body weight regulation.

Keywords: Arginine vasopressin, copeptin, AVPR1B, V1bR, BMI, diabetes

Introduction

Obesity is a partially heritable condition with a complex genetic basis and constitutes a major risk factor for diabetes mellitus (DM) [1-3]. Vasopressin (AVP), also called antidiuretic hormone, is a neurohypophyseal peptide involved in diverse physiological functions and released in conditions of hypotension and high plasma osmolality. AVP exerts antidiuretic effects through the vasopressin 2 receptor in the kidney [4], whereas the vasopressin 1a receptor (V1aR) is involved in platelet aggregation, vasoconstriction, liver gluconeogenesis and liver glycogenolysis [5-8], and the vasopressin 1b receptor (V1bR) is found in the pituitary gland and pancreas where it mediates secretion of ACTH, insulin and glucagon [9, 10].

An assay has been developed to measure plasma copeptin (copeptin), the C-terminal part of the AVP precursor. Copeptin is considered to be a reliable and clinically useful surrogate marker for AVP [11]. In the population based Malmö Diet and Cancer Study Cardiovascular Cohort (MDC-CC) we recently found that high copeptin, indicating over-activity of the AVP system, was independently associated with obesity, insulin resistance and risk of development of diabetes mellitus (DM) [12-14], and similar results has also been observed in a Dutch and a US population [15, 16]. However, whether these associations are causal or not is not known.

V1aR knock-out mice have a phenotype of elevated plasma glucose levels and fat-diet induced DM, as well as low triglyceride (TG) levels [17, 18]. We previously found that T-allele carriers of rs1042615 in the human V1aR gene (AVPR1A) gene have altered plasma glucose and TG levels, and an increased DM prevalence among those with a high fat intake [19], a phenotype strongly resembling the phenotype of the V1aR knock-out mice. Furthermore, the same polymorphism has been associated with elevated body mass index (BMI) in male T-allele carriers [20].

On the contrary, knock-out mice lacking the V1bR have a phenotype of low levels of glucose in plasma and better insulin sensitivity, as well as low levels of adrenocorticotrophic hormone and corticosterone, compared to wild type mice [21, 22], suggesting that overstimulation and/or enhanced endogenous activity of V1bR may result in a mild Cushing-like phenotype.

Here, we hypothesized that genetic variance of the human V1b receptor gene (AVPR1B) is associated with measures of obesity and with DM. Further, we tested whether genetic variation in the AVPR1B is associated with alterations of plasma copeptin level.

Materials and methods

Subjects

The Malmö Diet and Cancer study (MDC) is a population-based prospective cohort consisting of 30 447 subjects (DNA available on n=28 767) surveyed at a baseline examination in 1991-1996 [23, 24]. From this cohort, 6 103 subjects were randomly selected to be studied for the epidemiology of carotid artery disease (DNA available on n=6 027). This sample is referred to as the MDC cardiovascular cohort (MDC-CC) and was examined 1991-1994 [25]. At the MDC-CC baseline investigation, fasting plasma samples were obtained in 5 405 individuals and copeptin was measured. Furthermore, the MDC-CC was re-examined between 2007-2012 (67% participation rate) with fasting plasma samples and additional measurement of an oral glucose tolerance test (OGTT) [13].

The other part of MDC is referred to as the MDC replication cohort (n=24 344, DNA available on n=22 740). Fasting plasma samples were not obtained and copeptin was not measured in this cohort.

As genetic exposure is constant throughout life, we classified participants as having DM regardless of whether DM was established before or at the baseline exam or during a follow-up period of 14.0 ± 3.8 years after the baseline examination.

DM cases were defined based on six different national and regional DM registers: the Malmö HbA1c register (MHR) (see definition below), having a diagnosis of DM registered in the nationwide Swedish National Diabetes Register (NDR) [26] or the regional Diabetes 2000 register of the Scania region of which Malmö is the largest city [27], or the Swedish National Patient Register, which covers all somatic and psychiatric hospital discharges and Swedish Hospital-based outpatient care [28], or having DM as a cause of death in the Swedish Cause-of-Death Register [29], or having been prescribed anti-diabetic medication as registered in the Swedish Prescribed Drug Register [30].

The MHR analysed and catalogued all HbA1c samples at the Department of Clinical Chemistry taken in institutional and non-institutional care in the greater Malmö area from 1988 onwards. Individuals who had at least two HbA1c recordings ≥6.0% in the MHR using the Swedish Mono-S standardization system (corresponding to 7.0% according to the US National Glycohemoglobin Standardization Program [NGSP]) were considered as having DM.

In addition, DM at the baseline exam of MDC was obtained by self-report of a physician diagnosis or use of DM medication according to a questionnaire, or fasting whole blood glucose (which as described above was only available in the MDC-CC) of ≥ 6.1 mmol/L (corresponding to fasting plasma glucose concentration of ≥7.0 mmol/L). Furthermore, a DM diagnosis could be captured at the MDC-CC re-investigation by self-report of a physician diagnosis or use of DM medication according to a questionnaire or fasting plasma glucose of ≥ 7.0 mmol/L or a 120-min value post OGTT plasma glucose > 11.0 mmol/L. Finally, a DM diagnosis could be captured by fasting plasma glucose of ≥ 7.0 mmol/L which was analyzed in a re-investigation of about 1/3 of the MDC participants who also participated in the Malmö Preventive Project [31].

Anthropometric measures were measured at the MDC baseline examination. Abdominal obesity was defined as waist circumference > 102 cm in men and > 88 cm in women. Overweight was defined as BMI ≥ 25 kg/m^2, and obesity as BMI ≥ 30 kg/m^2.

Analyses in MDC-CC of overnight fasting glucose were carried out at the time of baseline and follow-up examination at the Department of Clinical Chemistry, Skane University Hospital in Malmö, which is attached to a national standardization and quality control system.

Copeptin was measured at baseline in MDC-CC in fasting plasma samples using a commercially available assay in the chemiluminescence/coated tube format (B.R.A.H.M.S AG, Hennigsdorf, Germany) as described previously [32]. Of those with available DNA, copeptin was measured in 5 195 individuals.

The study protocols were approved by the ethics committee of Lund University. All participants provided written informed consent.

Genotyping

DNA was extracted from frozen granulocyte or buffy coat with the use of QIAamp-96 spin blood kits (QIAGEN, Stockholm, Sweden) at the DNA extraction facility supported by SWEGENE. To analyze the AVPR1B polymorphism and capture the maximum of the genetic variance of AVPR1B, data from HapMap were used (www.hapmap.org) to select four tag single nucleotide polymorphisms (SNPs): rs35810727, rs28373064, rs35439639, and rs35608965. Primers and probes were custom synthesized by Applied Biosystems (Foster City, CA) according to standard recommendations for the AB Prism 7900HT analysis system, and genotyped with polymerase chain reaction-based TaqMan method [33].

Statistics

SPSS statistical software (version 20.0; SPSS Inc, Chicago, IL, USA) was used for all calculations. We used crude and multivariable adjusted logistic and linear regression to test if genetic variance was related to different measures of obesity, DM and copeptin. Copeptin was not normally distributed and was transformed using the natural logarithm. Additive genetic models were applied throughout. We used a combined variable of prevalent and incident DM in a logistic regression to test if genetic variance in rs35810727 was related to DM (regardless of whether DM was prevalent or incident). A two sided P-value of < 0.05 was considered statistically significant.

Results

The genotyping frequencies (Table 2a-d, Table 3) did not deviate from Hardy-Weinberg equilibrium (P>0.10 for all SNPs).

Measures of obesity

In MDC-CC, the major allele of rs35810727 was positively associated with BMI and overweight, both in a crude model and after adjustment for age and gender, whereas we did not find any significant association with obesity (Table 2a). Furthermore, the major allele of rs35810727 was associated with elevated waist circumference after adjustment for age and gender, though we did not find any association with abdominal obesity (Table 2a).

The association with BMI and overweight was replicated in the MDC replication cohort, whereas the association with waist was not significant in the MDC replication cohort (Table 3).

None of the other AVPR1B tag SNPs (rs28373064, rs35439639, rs35608965) were significantly associated with any measures of obesity (Table 2b-d).

Diabetes mellitus

We did not find any association between genetic variance in AVPR1B and DM in the MDC-CC (Table 2a-d). However, the genetic variance in rs35810727 was associated with DM in the MDC replication cohort (Table 3) and in the complete MDC cohort (n=30 447, OR (95% CI); 1.10 (1.00-1.20), P=0.04, model adjusted for age and gender). When BMI was added to the model, the association between DM and the major allele of rs35810727 remained in the MDC replication cohort (OR (95% CI); 1.15 (1.03-1.29), P=0.01) but not in the complete MDC cohort (OR (95% CI); 1.07 (0.97-1.17), P=0.18).

The AVPR1B tag SNPs rs28373064, rs35439639 and rs35608965 were not significantly associated with DM (Table 2b-d).

Copeptin

We tested the association between genetic variation in AVPR1B and copeptin in the MDC-CC subset and found that none of the tag SNPs were associated with altered copeptin level (in linear regression, after age and sex adjustment: rs35810727, P=0.72; rs28373064, P=0.87; rs35439639, P=0.74; and rs35608965, P=0.76).

Discussion

The key finding of this study is that genetic variance of AVPR1B is associated with elevated BMI and overweight. This association, which was discovered in the MDC-CC and replicated in the MDC replication cohort, implies a primary role for the AVP system in the pathophysiology of weight gain.

Variance in AVPR1B and measures of obesity

Our present data, linking elevated BMI and overweight to AVPR1B tag SNP rs35810727, are in line with our previous findings that imbalance of the AVP system, using copeptin as a proxy for AVP, is associated with measures of obesity both cross-sectionally and at follow up [13, 14]. Our current finding may, at least partially, be linked to an excessive activity of the AVP induced V1bR mediated ACTH release from the anterior pituitary gland, which, in contrast to the corticotropin releasing hormone induced ACTH release, has been reported to be resistant to glucocorticoid feedback [34]. One could speculate that carriers of the major allele of the AVPR1B tag SNP rs35810727 have enhanced AVPR1B signalling, either due to gain of V1bR receptor function, or enhanced gene expression. This would be expected to result in excessive V1bR mediated ACTH release. Overstimulation of the hypothalamic-pituitary-adrenal axis is linked to altered glucose and fat metabolism and development of overweight, obesity and insulin resistance [35], i.e. a Cushing-like phenotype.

Variance in AVPR1B and DM

The slightly higher overall frequency of DM (both prevalent and incident) in the MDC-CC than in the replication cohort was expected as the MDC-CC participants were screened for DM at baseline and reinvestigation with fasting glucose and OGTT (Table 1).

We did not find any association between variance in AVPR1B rs35810727 and DM in MDC-CC, whereas association was found in the MDC replication cohort and in the complete MDC cohort. These results may partly be explained by the different sizes of the cohorts. It can also be speculated that the register-based diagnoses of DM in the replication cohort, as opposed to the DM diagnoses primarily based on glucose-screening in the MDC-CC, would be more severe as it requires contact with the health care system. Assuming that the register-based DM diagnoses in the replication cohort represent more severe forms of DM, the association to the AVPR1B rs35810727 may be easier to detect.

Even though we are unable to draw any conclusions about association from these ambiguous results, the possible link between genetic variance in AVPR1B rs35810727 and DM are in line with previous findings that disturbance of the AVP system, measured as elevated copeptin levels, is an independent risk factor for DM development [12, 13, 16]. The suggested association, if any, between AVPR1B gene variance and DM, may partly be dependent upon AVPR1B mediated weight gain, as the significant association between rs35810727 and DM disappears after additional adjustment for BMI when the analysis is performed in the in the complete MDC cohort. However, the association remains after BMI adjustment when the analysis is performed solely in the MDC replication cohort. Anyhow, the ambiguous results with absence of association between rs35810727 and DM in the MDC-CC underline the need for replication of our DM finding.

Variance in the AVPR1B and copeptin

As we previously found association between elevated copeptin and measures of obesity in the MDC-CC [13, 14], we investigated the association between AVPR1B tag SNPs and copeptin level in the MDC-CC. Given the previous associations between elevated copeptin and measures of obesity in this cohort, the general complexity of the AVP receptor system, and the hypothesis of exaggerated V1bR signaling in carriers of the major allele of rs35810727, which may lead to compensatory changes in copeptin level, we did not know what to expect regarding copeptin level among subjects carrying the major allele of AVPR1B. The lack of association between rs35810727 of AVPR1B and plasma copeptin concentration suggests that elevated endogenous activity of the V1bR may explain the genetic association with overweight.

Strengths and weaknesses of the study

The MDC-CC is a large cohort, and we replicated parts of our findings in the even larger MDC replication cohort. Furthermore, our present data are supported by previous data linking disturbances of the AVP system to measures of obesity and DM.

We do acknowledge a number of limitations of our study. Our data suggests a link between the major allele of rs35810727 and DM. However, this association was not observed in the MDC-CC but only in the replication cohort and in the complete MDC cohort, why this finding needs to be replicated.

The four tag SNPs were selected according to HapMap data (www.HapMap.org) in order to capture the maximum of common genetic variation in the AVPR1B gene. Although the major allele of rs35810727 was associated with metabolic features that had previously been associated to dysregulation of the AVP system, we had no prior hypothesis of which of the four tag SNPs that would be associated with such traits, emphasizing the importance of our successful replication of our BMI findings, and the future replication of our DM findings.

Conclusion

Our finding strongly suggests that the major allele of AVPR1B is a genetic susceptibility factor for overweight. Furthermore, our data may indicate a link between AVPR1B variance and DM development. Altogether, this study supports a causal relationship between dysregulation of the pharmacologically modifiable AVP system and body weight regulation.

Acknowledgements

Conflict of interest

Dr Struck is employee of B.R.A.H.M.S GmbH which holds patent rights on the copeptin assay. The authors declare that there is no other duality of interest associated with this manuscript.

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Table 1. Population description.

	MDC cardiovascular cohort (n=6027)	MDC replication cohort (n=22740)
Gender (% men)	42.2	39.1
Age (years)	57.5 ± 5.9	58.2 ± 8.0
Body mass index (kg/m²)	25.8 ± 4.0	25.9 ± 4.0
Overweight (n, %)	3189 (53.0)	12255 (54.0)
Obesity (n, %)	806 (13.4)	3212 (14.2)
Waist circumference (cm)	84.2 ± 13.0	84.5 ± 15.2
Abdominal obesity (n, %)	913 (15.2)	3844 (16.9)
Diabetes mellitus at baseline (n, %)	540 (9.0)	977 (4.3)
Diabetes mellitus, new onset (n, %)[1]	570 (10.4)	2343 (10.8)
Copeptin (pmol/l)[2]	5.20 (3.22-8.24)	NA

Mean ± SD if nothing else specified. Baseline values if nothing else specified.
[1] after mean follow up time of 15.3 years in MDC cardiovascular cohort and 13.6 years in MDC replication cohort.
[2] expressed as median (interquartile range).
Abbreviations: MDC = Malmö Diet and Cancer Study

Table 2a. Malmö diet and cancer cardiovascular cohort: Genetic variation of AVPR1B in relation to metabolic phenotype.

Rs 35810727, N=5899, AA=39 (0.7%), AC=775 (13.1%), CC=5085 (86.2%). Genotyping success rate: 97.9%

Genotype	AA	AC	CC	P value[1]	P value[1,2]	Effect estimate[3]
BMI	25.4 ± 4.8	25.4 ± 4.0	25.8 ± 4.0	0.02	0.03	0.30 ± 0.14
Overweight (%)	46.2	47.7	53.7	0.001	0.004	1.23 (1.07-1.41)
Obesity (%)	15.4	12.0	13.6	0.35	0.37	1.10 (0.89-1.36)
Waist circumference	82.3 ± 13.7	83.0 ± 12.7	84.4 ± 13.0	0.003	0.03	0.78 ± 0.36
Abdominal obesity (%)	17.9	14.2	15.2	0.65	0.74	1.03 (0.85-1.26)
Diabetes mellitus (%) (prevalent or incident)	20.5	19.9	18.0	0.19	0.12	0.87 (0.73-1.04)

Continuous variables are given as means ± SD.
[1] Linear regression for continuous variables and logistic regression for dichotomous variables using additive model for the genetic effect.
[2] Model adjusted for age and sex.
[3] Beta-coefficient ± standard error for continuous variables, OR (95% CI) for dichotomous variables in model adjusted for age and sex.
Overweight = BMI ≥ 25; obesity: BMI ≥ 30; abdominal obesity: > 102/88 cm (men/women).

Table 2b. Malmö diet and cancer cardiovascular cohort: Genetic variation of AVPR1B in relation to metabolic phenotype.
Rs 28373064, N=5675, AA= 3967 (69.9%), AG= 1563 (27.5%), GG=145 (2.6%). Genotyping success rate: 94.2%

Genotype	AA	AG	GG	P value[1]	P value[1,2]
BMI	25.8 ± 4.0	25.7 ± 4.0	25.7 ± 3.9	0.71	0.79
Overweight (%)	53.1	52.6	49.0	0.45	0.53
Obesity (%)	13.4	13.1	15.2	0.94	0.92
Waist circumference	84.3 ± 13.1	83.9 ± 13.0	83.9 ± 12.3	0.37	0.53
Abdominal obesity (%)	15.3	14.5	17.2	0.79	0.84
Diabetes mellitus (%) (prevalent or incident)	17.9	18.6	20.7	0.39	0.33

Continuous variables are given as means ± SD.
[1] Linear regression for continuous variables and logistic regression for dichotomous variables using additive model for the genetic effect.
[2] Model adjusted for age and sex.
Overweight = BMI ≥ 25; obesity: BMI ≥ 30; abdominal obesity: > 102/88 cm (men/women).

Table 2c. Malmö diet and cancer cardiovascular cohort: Genetic variation of AVPR1B in relation to metabolic phenotype.
Rs 35439639, N=5905, GG=5697 (96.5%), AG= 205(3.5%), AA=3 (0.1%). Genotyping success rate: 98.0%

Genotype	GG	AG	AA	P value[1]	P value[1,2]
BMI	25.8 ± 4.0	25.5 ± 3.9	25.9 ± 3.7	0.33	0.33
Overweight (%)	52.8	55.1	66.7	0.45	0.42
Obesity (%)	13.5	10.7	0.0	0.21	0.20
Waist circumference	84.2 ± 13.0	83.1 ± 12.7	85.3 ± 17.1	0.26	0.34
Abdominal obesity (%)	15.1	15.6	33.3	0.68	0.69
Diabetes mellitus (%) (prevalent or incident)	18.5	15.1	0.0	0.17	0.17

Continuous variables are given as means ± SD.
[1] Linear regression for continuous variables and logistic regression for dichotomous variables using additive model for the genetic effect.
[2] Model adjusted for age and sex.
Overweight = BMI ≥ 25; obesity: BMI ≥ 30; abdominal obesity: > 102/88 cm (men/women).

Table 2d. Malmö diet and cancer cardiovascular cohort: Genetic variation of AVPR1B in relation to metabolic phenotype.
Rs 35608965, N=5841, TT=5233 (89.6%), CT=595 (10.2%), CC=13 (0.2%). Genotyping success rate: 96.9%

Genotype	TT	CT	CC	P value[1]	P value[1,2]
BMI	25.8 ± 4.0	25.9 ± 4.0	27.3 ± 3.9	0.33	0.36
Overweight (%)	52.7	54.6	61.5	0.31	0.34
Obesity (%)	13.3	13.8	30.8	0.40	0.43
Waist circumference	84.3 ± 13.0	84.1 ± 12.9	90.4 ± 11.1	0.84	0.85
Abdominal obesity (%)	15.4	14.5	23.1	0.71	0.68
Diabetes mellitus (%) (prevalent or incident)	18.4	18.0	23.1	0.92	0.89

Continuous variables are given as means ± SD.
[1] Linear regression for continuous variables and logistic regression for dichotomous variables using additive model for the genetic effect.
[2] Model adjusted for age and sex.
Overweight = BMI ≥ 25; obesity: BMI ≥ 30; abdominal obesity: > 102/88 cm (men/women).

Table 3. Malmö diet and cancer replication cohort: Genetic variation of AVPR1B in relation to metabolic phenotype.
Rs 35810727, N=21686, AA=136 (0.6%), AC=3106 (14.3%), CC=18444 (85.1%). Genotyping success rate: 95.4%

Genotype	AA	AC	CC	P value[1]	P value[1,2]	Effect estimate[3]
BMI	25.6 ± 3.8	25.7 ± 4.0	25.9 ± 4.1	0.01	0.003	0.21 ± 0.07
Overweight (%)	50.4	51.5	54.1	0.006	0.001	1.13 (1.05-1.21)
Obesity (%)	13.3	13.4	14.0	0.35	0.30	1.06 (0.95-1.17)
Waist circumference	84.1 ± 11.3	84.5 ± 19.1	84.4 ± 15.2	0.94	0.25	0.27 ± 0.007
Abdominal obesity (%)	14.1	15.6	16.9	0.045	0.03	1.12 (1.01-1.23)
Diabetes mellitus (%) (prevalent or incident)	14.7	12.1	14.2	0.005	0.002	1.18 (1.06-1.32)

Continuous variables are given as means ± SD.
[1] Linear regression for continuous variables and logistic regression for dichotomous variables using additive model for the genetic effect.
[2] Model adjusted for age and sex.
[3] Beta-coefficient ± standard error for continuous variables, OR (95% CI) for dichotomous variables in model adjusted for age and sex.
Overweight = BMI ≥ 25; obesity: BMI ≥ 30; abdominal obesity: > 102/88 cm (men/women).