The Adipocyte as a Novel TSH Target

A. Sorisky*, T.T. Antunes and A. Gagnon

Hormones Growth and Development Program, Ottawa Health Research Institute and Department of Biochemistry, Microbiology & Immunology, University of Ottawa, 725 Parkdale Avenue, Ottawa ON K1Y 4E9 Canada

Abstract: Thyroid stimulating hormone (TSH; also known as thyrotropin), binds cognate receptors on the surface of thyrocytes to regulate proliferation and thyroid hormone synthesis. This unidimensional view of TSH is being transformed as new evidence indicates that TSH acts on adipose tissue. Adipocyte inflammatory responses that predispose to cardiovascular disease may occur in thyroid disorders associated with elevated TSH levels.

Key Words: TSH, TSH receptor, adipocyte, interleukin 6, leptin, cardiovascular disease.

INTRODUCTION

An unexpected convergence has occurred recently between lines of research on 1) the role of thyroid stimulating hormone (TSH; also known as thyrotropin) with respect to thyroid gland function, and on 2) adipose tissue inflammation as a predisposing factor to cardiovascular disease (CVD). To understand the implications of these intersecting investigational directions, the role of TSH in the thyroid will be briefly reviewed, followed by a review of new information on TSH action on adipose cells.

TSH

TSH is a glycoprotein hormone belonging to a family that includes follicle stimulating hormone, luteinizing hormone, and human chorionic gonadotropin [1]. Members of this family are characterized by their heterodimeric structure. All members share a common subunit designated the alpha-sub-unit. The distinct effector function of each hormone therefore depends on the distinguishing attributes of their unique beta-subunits.

TSH is synthesized by cells in the anterior pituitary known as thyrotrophs, under the stimulatory influence of TSH releasing hormone released by the hypothalamus [2]. Once produced by thyrotrophs, TSH is secreted into the circulation to reach its principal target, the thyroid gland. It then acts on thyrocytes by specifically engaging its cognate receptor, the TSH receptor (TSHR) to induce cell proliferation, as well as thyroid hormone production and secretion.

TSHR STRUCTURE AND SIGNALING

The cell-surface TSHR belongs to the large superfamily of G protein-coupled receptors. The organization of the 7 transmembrane TSHR is complex. It has a large N-terminal extracellular ectodomain made up of many leucine-rich repeats, followed by a cysteine-rich flanking region that can be cleaved, creating A and B subunits that remain linked by disulfide bonds [3]. This receptor arrangement has been reported to be functional with respect to binding TSH and initiating intracellular signaling. As a result of receptor subunit associations during intracellular processing, as well as subunit shedding of the mature receptor, various multimer forms may be produced, but the physiological impact of each, if any, is not yet clear [4]. The fully processed, intact (i.e. not cleaved) holoreceptor is also competent for TSH signaling, and has been found in the thyrocyte as well as the adipocyte [5-7].

In thyrocytes, TSHR is coupled to adenylyl cyclase *via* Gs proteins (generating cAMP). Although still a somewhat controversial point, TSHR is also linked to phospholipase C (PLC) *via* Gq proteins [3, 4, 8, 9]. The cAMP pathway, acting through its main target, cAMP-dependent protein kinase (PKA), mainly controls thyrocyte proliferation and differentiation, as well as thyroid hormone secretion, whereas PLC has been proposed to regulate thyroid hormone synthesis [9]. It has been suggested that activated TSHR partitions into lipid rafts [10, 11], but other investigations found that TSHR signaling complexes do not localize in such structures [12]. TSHR signaling has been shown to be quenched by β-arrestin 2-dependent receptor internalization [13].

Many investigators have observed that TSHR also signals, to varying extent, through alternate pathways involving p38 MAPK, extracellular signal-regulated kinase 1 and 2 (ERK1/2; also known as p42/44 MAPK), phosphoinositide 3-kinase (PI3K), protein kinase B/Akt, and p70 S6 kinase, but the role of these novel signaling routes is less understood [3, 4, 6-9].

HYPOTHYROIDISM - SUBCLINICAL VERSUS OVERT

Subclinical hypothyroidism is a distinct, though related, clinical entity compared to overt hypothyroidism. It is a commonly occurring mild form of thyroid gland failure, often due to chronic autoimmune destruction, in which thyroid hormone production begins to falter. However, thyroid hormone output is able to be restored to normal levels due to an increase in TSH secretion from the pituitary (Fig. **1**). This compensated state may remain stable for years or eventually progress to frank thyroid gland failure i.e. overt hypothyroidism.

The overall prevalence of subclinical hypothyroidism is 1-10%, but rises to ~20% in the older population, in women

*Address correspondence to this author at Ottawa Health Research Institute, 725 Parkdale Avenue, Ottawa ON K1Y 4E9 Canada; Tel: 613-798-5555 #17572; Fax: 613-761-5036; E-mail: asorisky@ohri.ca

1389-5575/08 $55.00+.00

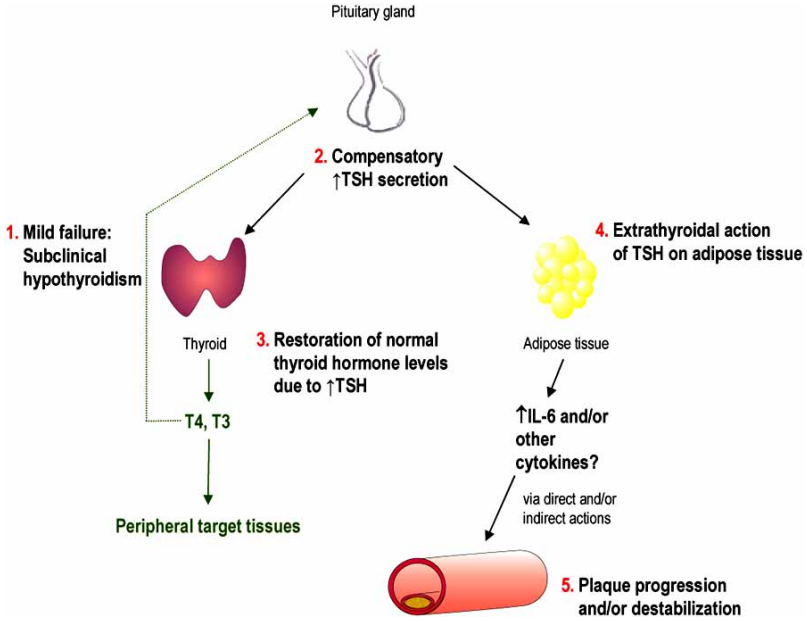

Fig. (1). Proposed mechanism through which subclinical hypothyroidism may elevate the risk of CVD.

more than men [14, 15]. The approach to treatment of subclinical hypothyroidism remains controversial [16, 17]. Briefly, some argue there is no actual disease state, since circulating thyroid hormones are kept in the normal range, albeit by an elevated TSH level. Proponents of this view hold that thyroid hormone therapy should be withheld, and only be used if/when overt thyroid gland failure develops. Others believe the compensated state of subclinical hypothyroidism does merit attention, and argue that thyroid hormone levels are actually not fully restored in this condition, even though they are in the normal laboratory range, and that thyroid hormone treatment is indicated from the outset.

An important and intriguing observation is that subclinical hypothyroidism itself has been independently associated with CVD. The well-established atherosclerotic risk caused by *overt* hypothyroidism (i.e. due to thyroid hormone deficiency) is a separate issue arising from different underlying mechanisms, and will not be discussed here [18]. In this review, our focus on the effect of TSH per se on pro-atherogenic adipocyte responses, independent of the additional variable of abnormally low thyroid hormone levels.

SUBCLINICAL HYPOTHYROIDISM AND CARDIOVASCULAR RISK

The Whickham Survey first showed a weak association between electrocardiographic changes and subclinical hypothyroidism [19], but the follow-up component did not show an increase in CVD [20], perhaps because participants received thyroid hormone therapy over time [18]. The Rotterdam study re-ignited the issue in 2000 [21]. Subclinical hypothyroidism occurred in ~11% of this population, and was significantly associated with aortic atherosclerosis and myocardial infarction (odds ratio 1.7 and 2.3 respectively), independent of age, body mass index, blood pressure, lipoproteins, smoking status, and immune reactivity. The attributable risk was similar to that of hypercholesterolemia, hypertension, smoking, or diabetes mellitus.

Several population studies subsequently examined the link between subclinical hypothyroidism and CVD. A New Mexico analysis of an elderly population (>65 years) reported a 2-fold higher prevalence of CVD with subclinical hypothyroidism [22]. A report from Nagasaki found subclinical hypothyroidism was associated with CVD in a cross-sectional analysis (4 fold more), and with higher 10 year mortality (2-fold) in a prospective study [23]. Danish investigators noted a 3-fold higher association of CVD with subclinical hypothyroidism [24]. A prospective English cohort study of patients with subclinical hypothyroidism found a 2-fold higher CVD mortality rate [25]. An Australian group found subclinical hypothyroidism was associated with CVD in a cross-sectional study (1.8 fold more) and 1.7 fold more in a longitudinal analysis [26].

A meta-analysis of studies from 1966-2005 concluded that subclinical hypothyroidism is associated with an elevated risk of CVD in observational studies (odds ratio 1.65, 95% confidence interval 1.28-2.12), with a similar trend noted in prospective studies (odds ratio 1.42, 95% confidence interval 0.91-2.21) [27]. The weight of the evidence clearly favours a linkage between subclinical hypothyroidism and CVD, yet a complete consensus is not in hand. Two American studies did not find an association of subclinical hypothyroidism with CVD, although a link with congestive heart failure was observed [28, 29]. The Leiden study of very old individuals (>85 years) found that subclinical, and surprisingly, also overt, hypothyroidism was linked to lower mortality in this special population [30, 31].

MECHANISTIC LINKS BETWEEN SUBCLINICAL HYPOTHYROIDISM AND CVD RISK

Clearly, the topic of subclinical hypothyroidism as an important CVD risk factor attracts significant attention in the cardiovascular and endocrine literature. Identifying mechanisms or mediators that could potentially elevate CVD risk in this condition may help to develop an improved clinical un-

derstanding. However, these entities remain elusive and do not appear to involve traditional CVD risk factors, as noted in the population studies above.

Recently, investigators have begun to address non-traditional risk factors through which subclinical hypothyroidism might lead to cardiovascular damage. Defective endothelium-dependent vasodilation, increased arterial stiffness, and insulin resistance have been observed with higher TSH levels [32-34]. Elevations in C-reactive protein (CRP), homocysteine, and free fatty acid (FFA) levels have been detected, suggesting a state of inflammation [35-37].

The mechanism underlying this inflammatory state has yet to be identified. The hypothesis that we introduce in this review is derived from the recognition that elevated circulating levels of TSH are capable of acting on adipocytes in culture (documented as TSHR-expressing cells), promoting a pro-inflammatory response. We have reported that TSH stimulates the release of the pro-atherogenic adipokine IL-6 from mouse and human differentiated abdominal adipocytes in culture [38-40]. TSH-mediated adipocyte inflammation may predispose to CVD (Fig. **1**). Others have reported that TSH also modulates leptin secretion from cultured adipocytes [41, 42].

INFLAMMATION AND CVD

Inflammatory processes are important in plaque initiation, progression, and destabilization [43-45]. Leukocyte adhesion depends on vascular endothelial cells upregulating expression and surface display of adhesion molecules, a process stimulated by modified lipoproteins, as well as cytokines such as IL-1β and tumour necrosis factor α (TNFα). Adherent monocytes/macrophages become foam cells, and secrete a variety of cytokines and chemokines that attract vascular smooth muscle cells and stimulate the formation of extracellular matrix within the plaque. Instability of the atherosclerotic plaque i.e. plaque disruption, an important predictor of vascular compromise and ischemia, is fueled by inflammation. Cytokines inhibit collagen production, weakening the matrix, and augment matrix metalloproteinases which degrade the protective fibrous cap of the plaque, which can then no longer sequester internal pro-thrombogenic lipids [43]. Circulating adipokines released from adipose tissue, such as IL-6, may influence plaque progression [46]. Some adipokines primarily act locally (paracrine or autocrine), and others, such as IL-6, can reach the general circulation [47, 48].

ADIPOSE TISSUE

Adipocytes modulate complex physiological processes (such as energy balance, carbohydrate and lipid metabolism, thrombosis, immunity, and inflammation) that influence cardiovascular and metabolic health. Dysfunctional adipose tissue, often linked to central obesity, increases the risk of CVD and type 2 diabetes. Low-grade inflammation and insulin resistance due to adipocyte dysfunction is thought to be a key event in promoting atherosclerosis [44, 45].

IL-6 AND CVD

IL-6 is secreted after N-terminal cleavage as a 184 amino acid peptide of ~23-32 kD depending on glycosylation [49]. Secreted from immune cells (macrophages, lymphocytes) and adipose cells, IL-6 acts on distant targets as well as in an autocrine/paracrine fashion [50]. IL-6 is the main regulator of hepatic production of CRP, a novel indicator of CVD risk [51]. CRP acts as a marker for the pro-atherogenic inflammatory state and may itself directly affect atherogenesis [52, 53]. Serum levels of IL-6 also predict long-term CVD events independently of CRP. The Physicians' Health Study showed that baseline IL-6 levels predict myocardial infarction in men [54]. Elevated IL-6 also identified women who developed CVD during the Women's Health Study [55]. A recent systematic review of studies published from 1999-2005 confirmed that IL-6 predicted the development of CVD with an adjusted relative risk between 1.1-3.1 [56]. Studies published in 2006 continue to demonstrate IL-6 is a significant prognostic indicator of CVD [57-59]. Therefore, IL-6 can increase the risk of CVD *via* direct actions on the vessel wall, or indirectly *via* induction of CRP.

IL-6 AND ADIPOSE TISSUE

Human studies estimate that whole body adipose tissue produces ~30% of circulating IL-6, and even more (up to 50%) with increased adiposity [47, 48, 60]. IL-6 in the venous drainage exceeds arterial levels across the adipose tissue bed, consistent with an endocrine "distant" role of IL-6 [47]. IL-6 is detected in adipose interstitial fluid by microperfusion technology in humans [61], and more IL-6 is secreted from abdominal omental than subcutaneous adipose tissue [62].

Genetic deletion of IL-6 in mice has yielded conflicting metabolic data. One group reported a phenotype of mature-onset obesity and insulin resistance, and suggested a defect in central regulation of energy expenditure as well as higher acylation stimulating protein levels [63, 64]. Another group showed that these same mice had normal adipose tissue function and did not develop mature-onset obesity [65]. Finally, acute IL-6 depletion with an anti-IL-6-neutralizing antibody improved insulin sensitivity in two obese mouse models [66]. More studies are needed to learn more about the impact of IL-6 deficiency, including adipose tissue-specific deletion of IL-6.

Various cell types within adipose tissue may secrete IL-6, such as adipocytes, preadipocytes, and macrophages. Human adipocytes account for ~10% of IL-6 secretion compared with adipose tissue in culture [62], but we have shown they, not preadipocytes, are the TSH-responsive cells [40]. Macrophages, found in human adipose tissue but only in the obese state, secrete the same amount of IL-6 as adipocytes [67, 68]. We have performed studies with murine J774 macrophage-like cells, and have not detected TSHR expression [7]. Similarly, TSH does not stimulate IL-6 release from human THP-1 macrophages (unpublished data). A contribution by endothelial cells within adipose tissue in response to TSH may be possible but has not been described [69].

Regulation of adipocyte IL-6 secretion is an emerging area of investigation. Modulation of constitutive IL-6 secretion occurs through mRNA expression, with changes in mRNA expression found to parallel secretion rates. In the sympathetic nervous system studies, β-adrenergic receptor-stimulated IL-6 release from cultured adipocytes was re-

ported to rise in response to isoproterenol [70]; this was seen in one *in vivo* human study but not another, using different methodologies [61, 71]. Glucocorticoids inhibit IL-6 secretion from adipose tissue [62, 70]. Interleukins (eg IL-1β), TNFα, and insulin can stimulate IL-6 release from adipocytes [62, 72, 73].

ADIPOSE TISSUE AND EXTRA-THYROIDAL TSH ACTION

TSH, secreted from thyrotrophs in the anterior pituitary gland, regulates thyroid gland growth, thyroid hormone synthesis, and secretion. Yet, dating back to work in the mid-60's on G proteins by the Nobel Prize co-winner Rodbell, there is evidence of extra-thyroidal effects of TSH on adipocytes [74]. Based on data from several laboratories, including our own [6, 7], it is now generally accepted that adipocytes express TSHR [3, 75, 76]. On the other hand, the network of TSHR signaling in adipocytes, and the downstream responses, remains to be fully investigated.

ADIPOCYTE MODELS USED FOR TSH STUDIES

Adipocyte research has been helped by the establishment of immortalized mouse cell lines that can be propagated and frozen as fibroblast-like preadipocytes, and then differentiated into rounded, lipid-filled adipocytes within 1 week [77]. The 3T3-L1 adipocyte line is used widely, as responses are robust (90-95% differentiation), reliable, and reproducible, and generally reflect behaviour of true adipocytes. Nevertheless, they are mouse-derived, aneuploid, and embryonic in origin, and so they are not a perfect model of primary diploid human preadipocytes.

Preadipocytes derived from the stromal fraction of human abdominal tissue can also be cultured and differentiated into adipocytes, permitting important anatomic-site comparisons (intra-abdominal omental versus sc depot) [78], as we have done [79-81], most recently with respect to TSH-stimulated IL-6 responses [40]. Human preadipocytes can be passaged a limited number of times (up to 3), and can differentiate into adipocytes with a modified differentiation protocol (including a peroxisome proliferator-activated receptor γ agonist) over 3 weeks; the 60-85% differentiation obtained with this protocol is ample for a stable adipocyte phenotype [40, 81]. Mature adipocytes can also be directly isolated from human adipose tissue; however, they are quite fragile (much more so than those from rodents), are short-lived in culture, cannot be propagated, and so are more challenging to study.

TSH SIGNALING AND ADIPOCYTE IL-6 RELEASE

IL-6 release is augmented by TSH in mouse 3T3-L1 as well as human abdominal sc adipocytes, and is regulated at the transcriptional level [38, 39]. TSH-dependent IL-6 secretion only occurs in 3T3-L1 differentiated adipocytes, and not preadipocytes [39]. Since the capacity for TSH to couple to adenylyl cyclase is only acquired upon 3T3-L1 adipocyte differentiation [7], IL-6 secretion may be cAMP-dependent. The cAMP-PKA pathway was implicated using the selective PKA inhibitor H89 (10 μM), which blocked TSH-stimulated IL-6 release from 3T3-L1 adipocytes [39]. Subsequent work with human abdominal sc and omental preadipocytes and adipocytes has shown that TSH stimulates IL-6 secretion only from differentiated adipocytes derived from the abdominal sc depot [40].

TSH-INDUCED INFLAMMATORY RESPONSES: *IN VIVO* HUMAN STUDIES

In order to study the extra-thyroidal action of TSH in humans, and in the context of normal thyroid hormone levels, the clinical paradigm of acute TSH exposure can be a useful first step. Thyroid cancer patients, as part of their treatment, have had thyroidectomies and radioablation of any residual thyroid gland tissue. For thyroid cancer recurrence screening, they remain on thyroid hormone replacement medication, and undergo a protocol in which they receive recombinant human (rh) TSH; any thyroid cancer cells that may have returned will "reveal" themselves (eg. by thyroglobulin levels in serum) since they respond to rhTSH. TSH levels rise during the 5 day test whereas thyroid hormone levels remain normal. A 3-fold increase in serum IL-6 was observed in 5 male patients following rhTSH administration and who had no evidence of thyroid cancer recurrence [40].

Other groups have used this novel human model system to study the acute extra-thyroidal effects of TSH. A larger study of 21 women and 3 men confirmed that TSH elevates IL-6 in this setting, and also reported an increase in TNFα [82]. Although viewed by some as a paracrine effector, TNFα, if elevated in the circulation, could also act as a pro-atherogenic factor [43-45].

Interestingly, impaired endothelium-dependent vasodilation has been seen in response to rhTSH administration, as well as in patients with subclinical hypothyroidism [32-34, 82].

These *in vivo* studies reveal that TSH acts in an extra-thyroidal manner to alter inflammatory cytokines and endothelial responses, but by their design, do not directly prove that the adipocyte is responsible.

SUMMARY

Recent developments reviewed here suggest a link between thyroid pathophysiology, inflammatory adipocyte-derived cytokines, and CVD. Epidemiological associations have identified an association between subclinical hypothyroidism, characterized by an isolated elevation of TSH levels, and CVD. Cellular and molecular studies have revealed that the TSHR is expressed in adipocytes, and when activated by TSH, results in IL-6 secretion, an inflammatory pro-atherogenic cytokine. *In vivo* human studies of acute TSH administration also demonstrate an inflammatory response that is generated by this hormone. This research expands our horizon of understanding interactions between the pituitary-thyroid axis, extra-thyroidal TSH actions on adipocytes, and CVD. Learning about this complex interplay holds the potential for improving our clinical strategy for a large population of individuals with subclinical hypothyroidism.

REFERENCES

[1] Szkudlinski, M. W.; Grossman, M.; Weintraub, B. D. *Curr. Opin. Endocrinol. Metab.*, **1997**, *4*, 354.

[2] Fliers, E.; Alkemade, A.; Wiersinga, W. M.; Swaab, D. F. *Prog. Brain Res.*, **2006**, *153*, 189.

[3] Davies, T. F.; Marians, R.; Latif, R. *J. Clin. Invest.*, **2002**, *110*, 161.
[4] Davies, T. F.; Ando, T.; Lin, R.-Y.; Tomer, Y.; Latif, R. *J. Clin. Invest.*, **2005**, *115*, 1972.
[5] Chen, C.-R.; Chazenbalk, G. D.; Wawrowsky, K. A.; McLachlan, S. M.; Rapoport, B. *Endocrinology*, **2007**, *148*, 3164.
[6] Bell, A.; Gagnon, A.; Grunder, L.; Parikh, S. J.; Smith, T. J.; Sorisky, A. *Am. J. Physiol.: Cell Physiol.*, **2000**, *279*, C335.
[7] Bell, A.; Gagnon, A.; Dods, P.; Papineau, D.; Tiberi, M.; Sorisky, A. *Am. J. Physiol.: Cell Physiol.*, **2002**, *283*, C1056.
[8] Rivas, M.; Santisteban, P. *Mol. Cell. Endocrinol.*, **2003**, *213*, 31.
[9] Van Sande, J.; Dequanter, D.; Lothaire, P.; Massart, C.; Dumont, J. E.; Erneux, C. *J. Clin. Endocrinol. Metab.*, **2006**, *91*, 1099.
[10] Latif, R.; Ando, T.; Daniel, S.; Davies, T. F. *Endocrinology*, **2003**, *144*, 4725.
[11] Latif, R.; Ando, T.; Davies, T. F. *Endocrinology*, **2007**, *148*, 3164.
[12] Costa, M. J.; Song, Y.; Macours, P.; Massart, C.; Many, M. C.; Costagliola, S.; Dumont, J. E.; Van Sande, J.; Vanvooren, V.; Saad, M. J. A. *Endocrinology*, **2004**, *145*, 1464.
[13] Frenzel, R.; Voight, C.; Paschke, R. *Endocrinology*, **2006**, *147*, 3114.
[14] Canaris, G. J.; Manowitz, N. R.; Mayor, G.; Ridgway, E. C. *Arch. Intern. Med.*, **2000**, *160*, 526.
[15] Hollowell, J. G.; Staehling, N. W.; Flanders, W. D.; Hannon, W. H.; Gunter, E. W.; Spencer, C. A.; Braverman, L. E. *J. Clin. Endocrinol. Metab.*, **2002**, *87*, 489.
[16] Gharib, H.; Tuttle, R. M.; Baskin, H. J.; Fish, L. H.; Singer, P. A.; McDermott, M. T. *J. Clin. Endocrinol. Metab.*, **2005**, *90*, 581.
[17] Surks, M. I. *J. Clin. Endocrinol. Metab.,* **2005**, *90*, 585.
[18] Cappola, A. R.; Ladenson, P. W. *J. Clin. Endocrinol. Metab.*, **2003**, *88*, 2438.
[19] Tunbridge, W. M.; Evered, D. C.; Hall, R.; Appleton, D.; Brewis, M.; Clark, F.; Evans, J. G.; Young, E.; Bird, T.; Smith, P. A. *Clin. Endocrinol.*, **1977**, *7*, 495.
[20] Vanderpump, M. P. J.; Tunbridge, W. M. G.; French, J. M.; Appleton, D.; Bates, D.; Clark, F.; Evans, J. G.; Rodgers, H.; Tunbridge, F.; Young, E. T. *Thyroid*, **1996**, *6*, 155.
[21] Hak, A. E.; Pols, H. A. P.; Visser, T. J.; Drexhage, H. A.; Hofman, A.; Witteman, J. C. M. *Ann. Int. Med.*, **2000**, *132*, 270.
[22] Lindeman, R. D.; Romero, L. J.; Schade, D. S.; Wayne, S.; Baumgartner, R. N.; Garry, P. J. *Thyroid*, **2003**, *13*, 595.
[23] Imaizumi, M.; Akahoshi, M.; Ichimaru, S.; Nakashima, E.; Hida, A.; Soda, M.; Usa, T.; Ashizawa, K.; Yokoyama, N.; Maeda, R.; Nagataki, S.; Eguchi, K. *J. Clin. Endocrinol. Metab.*, **2004**, *89*, 3365.
[24] Kvetny, J.; Heldgaard, P. E.; Bladbjerg, E. M.; Gram, J. *Clin. Endocrinol.*, **2004**, *61*, 232.
[25] Franklyn, J. A.; Sheppard, M. C.; Maisonneuve, P. *JAMA*, **2005**, *294*, 71.
[26] Walsh, J. P.; Bremmer, A. P.; Bulsara, M. K.; O'Leary, P.; Leedman, P. J.; Feddema, P.; Michelangeli, V. *Arch. Intern. Med.*, **2005**, *165*, 2467.
[27] Rodondi, N.; Aujesky, D.; Vittinghoff, E.; Cornuz, J.; Bauer, D. C. *Am. J. Med.*, **2006**, *119*, 541.
[28] Rodondi, N.; Newman, A. B.; Vittinghoff, E.; de Rekeneire, N.; Satterfield, S.; Harris, T. B.; Bauer, D. C. *Arch. Intern. Med.*, **2005**, *165*, 2460.
[29] Cappola, A. R.; Fried, L. P.; Arnold, A. M.; Danese, M. D.; Kuller, L. H.; Burke, G. L.; Tracey, R. P.; Ladenson, P. W. *JAMA*, **2006**, *295*, 1033.
[30] Gussekloo, J.; van Exel, E.; de Craen, A. J. M.; Meinders, A. E.; Frolich, M.; Westendorp, R. G. J. *JAMA*, **2004**, *292*, 2591.
[31] Cooper, D. A. *JAMA*, **2004**, *292*, 2651.
[32] Taddei, S.; Caraccio, N.; Virdis, A.; Dardano, A.; Versari, D.; L., G.; Salvetti, A.; Ferrannini, E.; Monzani, F. *J. Clin. Endocrinol. Metab.*, **2003**, *88*, 3731.
[33] Owen, P. J. D.; Rajiv, C.; Vinereanu, D.; Mathew, T.; Fraser, A. G.; Lazarus, J. H. *J. Clin. Endocrinol. Metab.*, **2006**, *91*, 2126.
[34] Fernandez-Real, J. M.; Lopez-Bermejo, A.; Castro, A.; Casamitjana, R.; Ricart, W. *J. Clin. Endocrinol. Metab.*, **2006**, *91*, 3337.
[35] Christ-Crain, M.; Meier, C.; Guglielmetti, M.; Huber, P.; Riesen, W.; Staub, J. J.; Muller, B. *Atherosclerosis*, **2003**, *166*, 379.
[36] Tuzcu, A.; Bahceci, M.; Gokalp, D.; Tuzun, Y.; Gunes, K. *Endocr. J.*, **2005**, *52*, 89.
[37] Caraccio, N.; Natali, A.; Sironi, A.; Baldi, S.; Frascerra, S.; Dardano, A.; Monzani, F.; Ferrannini, E. *J. Clin. Endocrinol. Metab.*, **2005**, *90*, 4057.
[38] Bell, A.; Gagnon, A.; Sorisky, A. *Arterioscler. Thromb. Vasc. Biol.*, **2003**, *23*, E65.
[39] Antunes, T. T.; Gagnon, A.; Bell, A.; Sorisky, A. *Obes. Res.*, **2005**, *13*, 2066.
[40] Antunes, T. T.; Gagnon, A.; Chen, B.; Pacini, F.; Smith, T. J.; Sorisky, A. *Am. J. Physiol.: Endocrinol. Metab.*, **2006**, *290*, E1140.
[41] Shintani, M.; Nishimura, H.; Akamizu, T.; Yonemitsu, S.; Masuzaki, H.; Ogawa, Y.; Hosoda, K.; Inoue, G.; Yoshimasa, Y.; Nakao, K. *Metabolism*, **1999**, *48*, 1570.
[42] Menendez, C.; Baldelli, R.; Camina, J. P.; Escudero, B.; Peino, R.; Dieguez, C.; Casanueva, F. F. *J. Endocrinology*, **2003**, *176*, 7.
[43] Libby, P. *Nature*, **2002**, *420*, 868.
[44] Libby, P. *Am. J. Clin. Nutr.*, **2006**, *83*(Suppl.), 456S-460S.
[45] Shoelson, S. E.; Lee, J.; Goldfine, A. B. *J. Clin. Invest.*, **2006**, *116*, 1793.
[46] Blaschke, F.; Takata, Y.; Caglayan, E.; Law, R. E.; Hsueh, W. A. *Arterioscler. Thromb. Vasc. Biol.*, **2006**, *26*, 28.
[47] Mohamed-Ali, V.; Goodrick, S.; Rawesh, A.; Katz, D. R.; Miles, J. M.; Yudkin, J. S.; Klein, S.; Coppack, S. W. *J. Clin. Endocrinol. Metab.*, **1997**, *82*, 4196.
[48] Kern, P. A.; Ranganathan, S.; Li, C.; Wood, L.; Ranganathan, G. *Am. J. Physiol. Endocrinol. Metab.*, **2001**, *280*, E745.
[49] Wong, P. K. K. *Arthritis Rheum.*, **2003**, *48*, 1177.
[50] Yudkin, J. S. *Int. J. Obes.*, **2003**, *27*(Suppl. 3), S25.
[51] Ridker, P. M.; Cannon, C. P.; Morrow, D.; Rifai, N.; Rose, L. M.; McCabe, C. H.; Pfeffer, M. A.; Braunwald, E.; Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI22) Investigators. *N. Engl. J. Med.*, **2005**, *352*, 20.
[52] Pepys, M. B.; Hirschfield, G. M. *J. Clin. Invest.*, **2003**, *111*, 1805.
[53] Pepys, M.; Hirschfield, G.; Tennent, G.; Gallimore, J.; Kahan, M.; Bellotti, V.; Hawkins, P.; Myers, R.; Smith, M.; Polara, A.; Cobb, A.; Ley, S.; Aquilina, J.; Robinson, C.; Sharif, I.; Gray, G.; Sabin, C.; Jenvey, M.; Kolstoe, S.; Thompson, D.; Wood, S. *Nature*, **2006**, *440*, 1217.
[54] Ridker, P. M.; Rifai, N.; Stampfer, M. J.; Hennekens, C. H. *Circulation*, **2000**, *101*, 1767.
[55] Ridker, P. M.; Hennekens, C. H.; Buring, J. E.; Rifai, N. *New Engl. J. Med.*, **2000**, *342*, 836.
[56] Lobbes, M. B. I.; Lutgens, E.; Heeneman, S.; Cleutjens, K. B. J. M.; Kooi, M. E.; van Engelshoven, J. M. A.; Daemen, M. J. A. P.; Nelemans, P. J. *Atherosclerosis*, **2006**, *187*, 18.
[57] Lee, K. W. J.; Hill, J. S.; Walley, K. R.; Frohlich, J. J. *Can. Med. Assoc. J.*, **2006**, *174*, 461.
[58] Fisman, E. Z.; Benderly, M.; Esper, R. J.; Behar, S.; Boyko, V.; Adler, Y.; Tanne, D.; Matas, Z.; Tenenbaum, A. *Am. J. Cardiol.*, **2006**, *98*, 14.
[59] Stork, S.; Feelders, R. A.; van den Beld, A. W.; Steyerberg, E. W.; Savelkoul, H. F. J.; Lamberts, S. W. J.; Grobbee, D. E.; Bots, M. L. *Am. J. Med.*, **2006**, *119*, 519.
[60] Yudkin, J. S.; Stehouwer, C. D. A.; Emeis, J. J.; Coppack, S. W. *Arterioscler. Thromb. Vasc. Biol.*, **1999**, *19*, 972.
[61] Orban, Z.; Remaley, A. T.; Sampson, M.; Trajanoski, Z.; Chrousos, G. P. *J. Clin. Endocrinol. Metab.*, **1999**, *84*, 2126.
[62] Fried, S. K.; Bunkin, D. A.; Greenberg, A. S. *J. Clin. Endocrinol. Metab.*, **1998**, *83*, 847.
[63] Wallenius, V.; Wallenius, K.; Ahren, B.; Rudling, M.; Carlsten, H.; Dickson, S. L.; Ohlsson, C.; Jansson, J.-O. *Nat. Med.*, **2002**, *8*, 75.
[64] Wernstedt, I.; Olsson, B.; Jernas, M.; Paglialunga, S.; Carlsson, L. M. S.; Smith, U.; Cianflone, K.; Wallenius, K.; Wallenius, V. *Endocrinology*, **2006**, *147*, 2690.
[65] Di Gregorio, G. B.; Hensley, L.; Lu, T.; Ranganathan, G.; Kern, P. A. *Am. J. Physiol. Endocrinol. Metab.*, **2004**, *287*, E182.
[66] Klover, P. J.; Clementi, A. H.; Mooney, R. A. *Endocrinology*, **2005**, *146*, 3417.
[67] Weisberg, S. P.; McCann, D.; Desai, M.; Rosenbaum, M.; Leibel, R. L.; Ferrante, A. W. J. *J. Clin. Invest.*, **2003**, *112*, 1796.
[68] Curat, C. A.; Wegner, V.; Sengenes, C.; Miranville, A.; Tonus, C.; Busse, R.; Bouloumie, A. *Diabetologia*, **2006**, *49*, 744.
[69] Donnini, D.; Ambesi-Impiombato, F. S.; Curcio, F. *Thyroid*, **2003**, *13*, 517.
[70] Path, G.; Bornstein, S. R.; Gurniak, M.; Chrousos, G. P.; Scherbaum, W. A.; Hauner, H. *J. Clin. Endocrinol. Metab.*, **2001**, *86*, 2281.

[71] Mohamed-Ali, V.; Flower, L.; Sethi, J.; Hotamisligil, G. S.; Gray, R.; Humphries, S. E.; York, D. A.; Pinkney, J. *J. Clin. Endocrinol. Metab.*, **2001**, *86*, 5864.
[72] Flower, L.; Gray, R.; Pinkney, J.; Mohamed-Ali, V. *Cytokine*, **2003**, *21*, 32.
[73] Fasshauer, M.; Klein, J.; Lossner, U.; Paschke, R. *Horm. Metab. Res.*, **2003**, *35*, 147.
[74] Birnbaumer, L.; Rodbell, M. *J. Biol. Chem.*, **1969**, *244*, 3477.
[75] Endo, T.; Ohta, K.; Haraguchi, K.; Onaya, T. *J. Biol. Chem.*, **1995**, *270*, 10833.
[76] Crisp, M. S.; Lane, C.; Halliwell, M.; Wynford-Thomas, D.; Ludgate, M. *J. Clin. Endocrinol. Metab.*, **1997**, *82*, 2003.
[77] Gregoire, F. M.; Smas, C. M.; Sul, H. S. *Physiol. Rev.*, **1998**, *78*, 783.
[78] Montague, C. T.; O'Rahilly, S. *Diabetes*, **2000**, *49*, 883.
[79] Bell, A.; Grunder, L.; Sorisky, A. *Obes. Res.*, **2000**, *8*, 249.
[80] Shahparaki, A.; Grunder, L.; Sorisky, A. *Metabolism*, **2002**, *51*, 1211.
[81] Constant, V. A.; Gagnon, A.; Landry, A.; Sorisky, A. *Diabetologia*, **2006**, *49*, 1402.
[82] Dardano, A.; Ghiadoni, L.; Plantinga, Y.; Caraccio, N.; Bemi, A.; Duranti, E.; Taddei, S.; Ferrannini, E.; Salvetti, A.; Monzani, F. *J. Clin. Endocrinol. Metab.*, **2006**, *91*, 4175.

Received: 24 November, 2006 **Revised: 28 May, 2007** **Accepted: 29 May, 2007**