Recent data on the regulation of lipolysis by catecholamines and natriuretic peptides

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The process of adipose tissue lipolysis, i.e. the catabolic process leading to the breakdown of fat cell triacylglycerols (TAG) into fatty acids and glycerol, delivers non esterified fatty acids (NEFA) to circulation where NEFAs serve as the major circulating lipid fuel. This highly regulated process usually provides adjusted amounts of NEFAs to fat oxidizing tissues. Abnormalities of NEFA fluxes have been observed in some forms of obesity. Particularly, upper-body obesity results in several abnormalities of systemic NEFA availability that could contribute to the adverse health consequences usually described in this form of obesity. Excess NEFA availability can induce insulin resistance with respect to muscle glucose uptake, suppression of endogenous glucose production, hypertriglyceridemia and promote reduction of hepatic insulin clearance and impaired insulin secretion.

The wide range of plasma NEFA availability is essentially related to the sensitivity of adipose tissue lipolysis. Adipose tissue lipolytic activity is regulated by the balance between hormones that stimulate (primarily catecholamines) and those that inhibit hormone sensitive lipase (HSL) (insulin). In vitro studies have revealed regional differences in the antilipolytic effects of insulin; however it is still unclear whether these differences are fully operative in vivo. Regional heterogeneity of insulin-regulated NEFA release in vivo has recently been reported [30]. Sedentary obese women have whole body resistance to the suppression of lipolysis by insulin. Intra-abdominal adipose tissue may be the site of resistance since insulin resistance was not evident in abdominal and femoral subcutaneous adipose tissue (local antilipolytic responsiveness was investigated with in situ microdialysis) [13]. Catecholamines are important stimulators of NEFA release under conditions of stress and during exercise. In vitro studies have revealed the complexity of catecholamine action on the human fat cell. Epinephrine and norepinephrine, stimulate and/or inhibit lipolysis depending on their relative affinity for adrenergic receptor subtypes, the relative number of fat cell β- and α₂-adrenergic receptors expressed in the fat cell and their coupling efficiency to heterotrimeric G proteins involved to the transduction of the signal (Gs and Gi-protein, respectively). Heterogeneity of human adipocytes responsiveness towards catecholamines is well established. Functional differences could be related to adrenergic receptors by themselves or to various other downstream elements of the lipolytic cascade (Gs/Gi proteins, protein-kinase A or hormone sensitive lipase) [19, 21]. Concerning β-adrenergic responsiveness, in vitro assays have clearly shown that human fat cell lipolysis is essentially regulated by β₂- and β₁-adrenergic receptor stimulation [28]. No evidence could be provided for a β₂-adrenergic receptor-dependent lipolysis during isoprenaline, norepinephrine or epinephrine stimulation of human fat cells in vitro [28, 44]. It is now well established that CGP12177, the drug usually used to assess a β₂-adrenergic effect in human fat cells is acting, in fact, via an atypical state of the β₁-adrenoceptor when this compound is used at higher concentrations. The atypical state of the β₂-adrenergic receptor contributes to the mediation of the stimulatory effects induced by non-conventional partial agonists such as (-) CGP12177 [17, 18]. Confirmation of the lack of β₂-adrenergic effect in humans has also been provided by in vivo studies. During isoprenaline infusion at dosages < 200 ng/kg min there was no evidence for a β₂-adrenergic receptor-mediated increase in human lipolysis, energy expenditure and lipid oxidation [36]. Similar conclusions were obtained when using in situ microdialysis to delineate the various β-adrenergic receptor subtypes involved in the control of subcutaneous adipose tissue lipolysis [1]. Concerning recent genetic approaches, a number of controversial results, which cannot be listed here, have been published concerning the existence of a polymorphism in codon 64 (Trp64Arg) of the β₂-adrenergic receptor gene and the development of obesity and obesity-related disorders.

Studies performed in adults with long-standing obesity suggest a reduced lipolytic sensitivity to catecholamines in subcutaneous abdominal adipose tissue. Profound unresponsiveness of the subcutaneous adi-
pose tissue to neurally stimulated lipolysis has been described in obese subjects [7]. Reduced \( \beta_2 \)-adrenergic lipolytic responsiveness has been reported in fat cells from obese subjects [35] or from subjects with a reduced isoprenaline sensitivity [24]. In addition, an increased antilipolytic responsiveness linked to \( \alpha_2 \)-adrenergic receptor stimulation has also been found in subcutaneous adipocytes of obese of both sexes [29]. The lipolytic defects have been confirmed in in vivo studies [2, 3, 15]. Using in situ microdialysis, a specific impairment in the capacity of \( \beta_2 \)-adrenergic receptors agonists to promote lipolysis has been reported in the subcutaneous abdominal adipose tissue of obese adolescent girls [8]. Moreover, when performing i.v. administration of selective \( \beta_1 \)- and \( \beta_2 \)-adrenergic receptor-agonists, the increase in lipolysis and thermogenesis promoted by a selective \( \beta_2 \)-adrenergic stimulation (salbutamol) was reduced in obese subjects while \( \beta_2 \)-adrenergic receptor-mediated (dobutamine) metabolic processes (i.e. lipolysis, thermogenesis and lipid oxidation) were similar in obese and lean men. In conclusion, \( \beta_2 \)-adrenergic-mediated increases in thermogenesis and lipid oxidation are impaired in the obese. It is suspected that a dysfunction of the \( \beta_2 \)-adrenergic pathway or of the \( \beta_2 \)-adrenergic receptor density may play a role in the etiology or maintenance of a relatively increased fat mass and consequently obesity [38]. However, it is unknown whether, in parallel, \( \beta_2 \)-adrenergic receptor density or coupling is reduced in skeletal muscle cells or blood vessels in obese subjects. It is an important point which requires further investigations. A dominant expression of the beta2-adrenergic receptor subtype, that could be of muscular or vascular origin, exist in skeletal muscle homogenates [23]. When performing a microdialysis approach in human skeletal muscle, it is only the \( \beta_2 \)-adrenergic receptor subtype which is of importance for the regulation of lipolysis and the control of local blood flow [11]. Thus, a reduction of \( \beta_2 \)-adrenergic efficacy, if occurring, might provide an explanation for the reduced response in lipid oxidation and thermogenesis.

Further evidence for a putative role of \( \beta_2 \)-adrenergic receptors in the etiology of obesity is also provided by the discovery of three recently described polymorphisms (the Gln27Glu, Arg16 Gly variants and two polymorphic sites T → C substitution at −47 and T → C substitution at −20, located in the 5' leader cistron) of the \( \beta_3 \)-adrenergic receptor gene that are associated with obesity [16, 22, 31, 34, 45]. Interestingly, physical activity was able to counterbalance the effect of the genetic predisposition to obesity in such subjects [32]. Full \( \beta_2 \)-adrenergic activation of human fat cell usually requires synergistic activation of \( \beta_1 \)- and \( \beta_2 \)-adrenergic receptors. Even if \( \beta_1 \)-adrenergic effects are found to be preserved in obese subjects, a \( \beta_2 \)-adrenergic defect could be sufficient to alter the normal \( \beta \)-adrenergic responsiveness. In addition, in human fat cells, any reduction of \( \beta_2 \)-adrenergic receptor-mediated lipolytic response will disturb the normal functional balance existing between \( \alpha_2 \)- and \( \beta \)-adrenergic receptor-mediated effects and amplify the reduction of the lipolytic responsiveness initiated by the physiological amines in stressfull situations.

In vitro assays in isolated human fat cells have clearly delineated variations of human fat cell \( \alpha_2 \)-adrenergic receptors function and expression in various physiological and pathological situations [20]. In human fat cells where \( \alpha_2 \)-adrenergic receptors outnumber \( \beta \)-adrenergic receptors, the preferential recruitment of the \( \alpha_2 \)-adrenergic receptors at the lowest catecholamine concentrations inhibits lipolysis [26]. The strongest \( \alpha_2 \)-adrenergic effect has been observed in the adipocytes from subcutaneous adipose tissue from men and women ; \( \alpha_2 \)-adrenergic receptors are particularly expressed in subcutaneous adipocytes from obese subjects [25, 29]. Moderate weight loss, leads to a higher adipose cell lipolytic efficiency which is associated with changes at receptor levels (mainly increased \( \beta_2 \)- and decreased \( \alpha_2 \)-sensitivities) [27].

Results of our group, based on the utilization of in situ microdialysis technique, provide a response to an old question concerning a putative role of fat cell \( \alpha_2 \)-adrenergic receptors in the physiological control of lipid mobilization. Initial studies, performed in normal subjects, and based on the administration of an \( \alpha_2 \)-agonist (clonidine) or catecholamines, directly in the microdialysis probe, have not been fully conclusive to attribute a physiological role to these receptors [9, 33]. In search for more relevant physiological protocols, exercise was selected to promote a calibrated activation of sympathetic nervous system. These studies have focused attention on the involvement of epinephrine in the control of lipid mobilization through activation of antilipolytic \( \alpha_2 \)-adrenergic receptors in human subcutaneous adipose tissue during exercise [42]. The \( \alpha_2 \)-adrenergic effect was observed when plasma epinephrine levels have been increased consecutively to an increased stressfull situation. Moreover, exercise-induced lipolysis is impaired in subcutaneous adipose tissue in obese men ; the physiological stimulation of adipocyte \( \alpha_2 \)-adrenergic receptors during exercise contributes to this impairment. The blunting of lipid mobilization was suppressed by local administration of an \( \alpha_2 \)-adrenergic receptor antagonist [43]. Striking differences were observed depending on the extent of fat deposits and the intensity of exercise. In heavily trained men, it was impossible to reveal any \( \alpha_2 \)-adrenergic effect in the reduced subcutaneous fat deposits of such subjects [6]. Conversely to the results obtained in obese men, in subcutaneous abdominal adipose tissue of obese women, \( \alpha_2 \)-adrenergic receptors...
appeared less involved in the control of the lipolytic process during a physiological stimulation of SNS promoted by exercise. Gender differences in the adrenergic regulation of lipid mobilization have also been reported in non-obese patients performing short submaximal exercise bouts; however the nature of the differences has not been interpreted by the authors [12]. The noticeable difference between the two genders seems to be related to the profile of SNS and adrenal medulla activation promoted by exercise. Exercise-induced increments of plasma norepinephrine were quite similar in both sexes while plasma epinephrine levels were practically unchanged in obese or non-obese women while being noticeably increased in men. It could be hypothesized, that activation of fat cell β2-adrenergic receptors by exercise mainly occurs when epinephrine release occurs. It could be proposed that exercise-related activation of adrenal medulla and epinephrine release probably represents a negative stress for optimal lipid mobilization. Attenuated stress-induced increases in plasma epinephrine in women suggest that females are less sensitive and/or less responsive to adrenal medullary activation. This is supported by findings of gender differences in adrenal medullary catecholamine content, release and degradation [14]. The functional studies, based on microdialysis investigation of local lipid mobilization in subcutaneous adipose tissue, suggest that, when looking for optimizing exercise-induced lipid mobilization it will be important to maximize positive stress (norepinephrine release) while minimizing negative stress (epinephrine release).

In humans, in addition to catecholamines, parathyroid hormone, cortisol and growth hormone are also able to promote stimulation of lipolysis, nevertheless they appear to be less potent than catecholamines. A novel pathway for stimulation of lipolysis has been characterized in human fat cells [39]. Natriuretic peptides, specially the atrial natriuretic peptide (ANP), stimulate lipolysis through a cGMP-dependent (cAMP-independent) pathway with a relative order of potency of the peptides which is ANP>BNP>CNP; the effect of CNP being very weak. A type A ANP receptor, with its guanylyl cyclase activity, is present in human fat cells. To search for an ANP action in vivo and a putative influence of obesity on ANP responsiveness, we compared the lipolytic effects of human ANP (h-ANP) on isolated subcutaneous abdominal adipose tissue fat cells from young, healthy lean and obese men. Moreover, the lipid mobilizing effects of an intravenous infusion of h-ANP was studied as well as various metabolic and cardiovascular parameters. h-ANP (50 ng/min/kg) was infused i.v. for 60 min. Microdialysis probes were inserted in subcutaneous adipose tissue to measure modifications of the extracellular glycerol concentrations during h-ANP infusion. h-ANP induced a marked and similar increase in glycerol, NEFAs and a weak increase in plasma insulin levels in lean and obese. Plasma norepinephrine concentrations rose similarly during h-ANP infusion in lean and obese subjects. The effects of h-ANP infusion on the autonomic nervous system were similar in both groups. In SCAAT, h-ANP infusion increased extracellular glycerol concentration and decreased local blood flow similarly in both groups. The increase in extracellular glycerol observed during h-ANP infusion was not modified when 0.1 mmol/L propranolol (non-selective β-antagonist) was added to the microdialysis probe perfusate to prevent β-adrenergic receptor activation [10]. These data show that ANP is a potent lipolytic hormone independent of the weak activation of the sympathetic nervous system promoted by h-ANP infusion. Moreover obesity did not modify the lipolytic and lipid mobilizing effect of ANP in young obese subjects.

**CONCLUDING REMARKS AND PERSPECTIVES**

Although the knowledge of cellular events involved in the regulation and dysregulation of lipolysis by catecholamines and other lipolytic agents have considerably been extended, the exploration of the regulatory processes in vivo remain difficult. Whole body and regional investigations of lipolytic processes have been improved by the concomitant development of in situ microdialysis, measurement of arterio-venous differences in abdominal subcutaneous adipose tissue and stable isotopes techniques. Because of its anatomical location, the direct assessment of the metabolic activity of intraabdominal fat is not easily feasible in humans. It is probable that, in the near future, concomitant assessment of regional lipolysis in subcutaneous adipose tissue by in situ microdialysis and of the whole body lipolysis by stable labeled isotope kinetics will facilitate insight into the relative contribution of subcutaneous adipose tissues and other adipose tissues (presumably intraabdominal and intramuscular depots). The recent results of the group focus attention upon various factors which must be taken in account when exercise is prescribed to obese patients to promote lipid mobilization in the various fat deposits. Obese subjects seem to have an impaired utilization of NEFA in the muscle [4], and β-adrenergic stimulation of lipolysis and fat oxidation are impaired in obese [40]. However, increase in plasma NEFA concentration leads to similar increases in lipid oxidation and energy expenditure in obese and lean men [37]. The accumulation of fat in obese subjects may therefore more likely be due to a defect in adipose tissue lipolysis than a defect in lipid oxidation; the subcutaneous deposits being usually more resistant to catecholamine-in-
ducid lipolysis due to the high level of expression of α2-adrenergic receptors and which could also be linked, depending on the patients, to a possible dysregulation of β2-adrenergic receptor function. Management of β-adrenergic responsiveness is not easy if the defect is related to β2-adrenergic receptor dysfunction. Nevertheless, physical training is known to improve β-adrenergic responsiveness in subcutaneous adipose tissue [5, 41]. In addition, pharmacotherapy based on administration of α2-adrenergic receptor antagonists to improve lipolysis can be proposed to support short-term induction of lipid mobilization during early or late-stages of a weight loss program associating suitably calibrated exercise bouts and reasonable caloric restriction for an expected long-term weight maintenance. Concerning ANP/BNP-related questions, it is still necessary to provide additional physiological or pathological validation of ANP/BNP involvement in the regulation of lipid mobilization in situations related to physiological or altered pathological release of these hormones. Moreover it also remains to establish if this kind of new lipolytic agent, having beneficial actions on blood pressure and natriuresis, could offer some interest in lipid mobilizing processes aiming at the improvement of lipid mobilization, energy expenditure and lipid oxidation.

REFERENCES


