Chemistry & Biology 13, 1019–1027, October 2006 DOI 10.1016/j.chembiol.2006.09.010

Small-Molecule Compounds that Modulate Lipolysis in Adipose Tissue: Targeting Strategies and Molecular Classes

Review

Minghan Wang[1,*] and Christopher Fotsch[2,*]
[1]Department of Metabolic Disorders
[2]Chemistry Research and Development
Amgen Incorporated
Thousand Oaks, California 91320

Lipolysis is an important pathway in maintaining energy homeostasis through the degradation of triglycerides in adipose tissue and the release of fatty acids into the circulation as an energy source. However, an elevated level of circulating fatty acids leads to unfavorable metabolic effects such as insulin resistance and dyslipidemia. Cell surface receptors and intracellular components of the lipolytic pathway have been targeted to develop antilipolytic agents, among which are G-protein-coupled receptor agonists and lipase inhibitors. In addition, molecules that stimulate lipolysis have been tested in clinical trials as a treatment for obesity. Together, these molecules represent a diverse group of regulators for this pathway. This review will discuss strategies to target lipolysis and the major issues with representative small-molecule modulators of this pathway.

Introduction

Energy homeostasis is balanced by food intake and energy expenditure. When energy intake exceeds utilization, the adipose tissue serves as a reservoir for energy storage in the form of triacylglycerol (TG). Under fasting conditions or during periods of increased energy demand, the degradation of TG occurs in adipose tissue to release free fatty acids (FFAs) into the circulation as an energy source for other tissues. FFAs are mobilized from adipose tissue as a result of the balance between lipolysis and re-esterification. This dynamic process is essential for systemic lipid metabolism and energy homeostasis (Figure 1). Disturbances in fatty acid metabolism have been linked to insulin resistance and other features of the metabolic syndrome. Elevated plasma FFA levels have been observed in both mild and severe type 2 diabetics [1], and individuals with higher plasma fasting FFAs have an increased risk of type 2 diabetes [2, 3]. Since FFAs are immediate precursors of hepatic TG synthesis [4], elevated plasma FFA levels are one of the primary causes of hyperlipidemia. Increased plasma FFAs also augment basal hepatic gluconeogenesis [5, 6], inhibit insulin-dependent glucose disposal [7–9], and impair microvascular function [10]. In addition, chronic FFA exposure inhibits glucose-dependent insulin secretion by isolated human pancreatic islets [11]. One of the direct molecular defects caused by elevated plasma FFAs is the accumulation of intracellular lipids, which impairs insulin signaling, resulting in hepatic and peripheral insulin resistance [12].

*Correspondence: mwang@amgen.com (M.W.), cfotsch@amgen.com (C.F.)

FFAs are mobilized from adipose tissue via lipolysis. Two lipolytic enzymes, hormone-sensitive lipase (HSL) [13] and adipose triglyceride lipase (ATGL), also known as desnutrin or calcium-independent phospholipase A2ζ (iPLA2ζ) [14–17], play central roles in the degradation of TG. In addition, HSL also hydrolyzes diacylglycerol (DG) [15]. The activities of HSL and ATGL are highly regulated. The activation of HSL is mediated by phosphorylation and translocation [13], and the expression of ATGL is stimulated by fasting and suppressed upon refeeding [15, 16]. These molecular mechanisms mediate the hormonal regulation of lipolysis to accommodate the need for energy demand or storage [18], so that the lipolytic rate is synchronized with the metabolic status. Dysregulation of lipolysis could lead to elevated plasma FFAs and cause insulin resistance. Increased lipolysis has been observed in both lean type 2 diabetics and obese subjects [19, 20]. The lipolysis in these subjects is less responsive to suppression by insulin [19, 20]. In addition to the deleterious effects of elevated plasma FFAs, increased lipolysis supplies extra glycerol to augment gluconeogenesis [21]. Therefore, inhibition of lipolysis may be a viable approach to reduce plasma FFAs and improve insulin sensitivity in patients with type 2 diabetes. This concept is supported by the clinical use of niacin and its analogs (i.e., acipimox), which exert their pharmacologic effects primarily by inhibiting lipolysis [22]. Acipimox improves hepatic and peripheral insulin sensitivity by reducing plasma FFAs without increasing adipokine secretion [23], suggesting that reducing plasma FFAs alone is sufficient to treat insulin resistance. Niacin reduces pro-atherogenic lipoproteins and elevates high-density lipoprotein (HDL) in humans [22]. Niacin and its analogs also improve insulin action in short-term treatments [23–26]. However, the clinical use of niacin is limited due to side effects, mainly skin flushing mediated by prostaglandin release [22, 27, 28]. A sustained-release form of niacin is effective in ameliorating flushing, but is associated with hepatic toxicity [29]. Niacin also induces significant insulin resistance after long-term use and, at high doses, causes deterioration of glycemic control [30, 31]. Based on these clinical findings, there is a clear medical need for improved antilipolytic treatments.

The Lipolysis Pathway

The FFA release in the cell involves the hydrolysis of TG and subsequent degradations of DG and monoacylglycerol (MG) (Figure 2). ATGL and HSL are the main lipolytic enzymes for the initial steps, and monoglyceride lipase (MGL) converts MG to FFA and glycerol in the final step (Figure 2). Carboxylesterase 3 and two calcium-independent phospholipase A_2 ($iPLA_2$) members also have TG lipase activity [17, 32], but their contribution to the fat cell lipolysis remains to be determined. ATGL is the predominant TG lipase expressed in white adipose tissue [15–17]. ATGL is localized on the surface of the lipid droplet. The expression of ATGL is upregulated by fasting and glucocorticoids [15, 16], suggesting that ATGL might be involved in the activation of lipolysis.

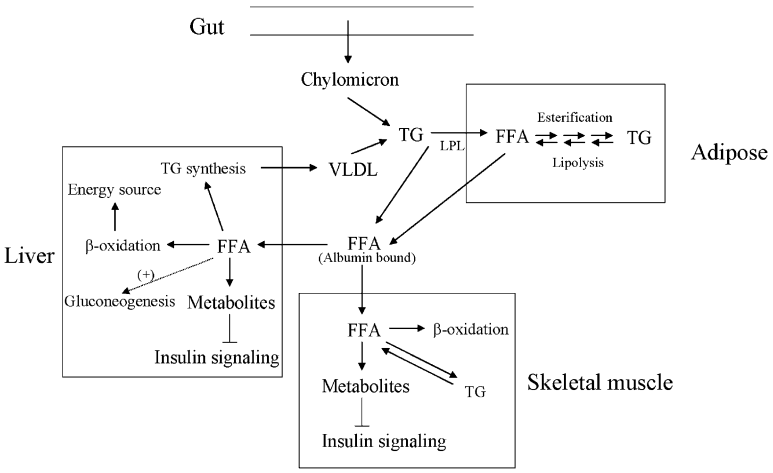


Figure 1. FFA Metabolism

The TG contents carried by chylomicron and VLDL particles are hydrolyzed by lipoprotein lipase (LPL) located on the endothelium and adipocytes releasing FFAs, which enter the fat cell via facilitated uptake for fat storage. A fraction of the released FFAs enter the blood circulation. The plasma FFAs are important for hepatic lipid synthesis and gluconeogenesis. FFAs are also oxidized as an energy source in multiple tissues. The accumulation of TG in both liver and skeletal muscle results in the increased fatty acyl metabolites that cause insulin resistance [70]. For simplicity, some components in the FFA metabolism pathway are omitted, and only liver and skeletal muscle are shown here as the major FFA utilizing organs.

Human genetic studies suggest that ATGL is associated with plasma FFA concentrations and risk of type 2 diabetes [33], highlighting the important role of lipolysis in lipid metabolism and insulin sensitivity. HSL is mainly a DG lipase with significant TG lipase activity [34–36]. The hormonal activation of lipolysis is mediated by the intracellular levels of cAMP and cGMP, which activate protein kinase A (PKA) and protein kinase G (PKG), respectively (Figure 2). These kinases phosphorylate HSL promoting the translocation of HSL from the cytosol to the lipid droplet where TG hydrolysis occurs. This process is also facilitated by the phosphorylation of perilipin A, a structural protein on the surface of the lipid droplet (Figure 2). Upon phosphorylation, perilipin A dissociates from the lipid droplet and allows HSL access to the core lipids [13, 37]. While perilipin knockout in mice resulted in leanness [38], reduced perilipin levels are associated with increased lipolysis in human obesity [39, 40]. The intracellular FFAs produced by lipolysis are bound to adipocyte fatty-acid-binding protein (FABP), which helps solubilize and transport FFAs out of the cell. FABP is physically associated with HSL and relieves product inhibition by binding to FFAs released from HSL [41, 42]. Consistent with this role, FABP deficiency decreases lipolysis and increases fat mass [43, 44].

Molecular and Physiological Regulation of Lipolysis

Lipolysis is regulated by hormones such as catecholamines and insulin. Catecholamines interact with both Gi-G-protein-coupled α_2-adrenergic receptor (α_2-AR) and Gs-G-protein-coupled β-adrenergic receptors (β_1-, β_2-, and β_3-ARs), and regulate intracellular cAMP levels in opposite directions [45] (Figure 2). Normally the lipolytic effect of β-ARs dominates, and the net effect of catecholamine action is stimulation of lipolysis. But the balance could shift under certain physiological conditions, which may be associated with alterations of the available catecholamine concentrations [45]. Insulin suppresses lipolysis through the phosphorylation and activation of phosphodiesterase 3B (PDE3B), which hydrolyzes both cAMP and cGMP [46, 47]. PDE3B is the main PDE involved in the lipolysis in human adipocytes [48]. There are a number of other molecules that exert antilipolytic effects through the activation of Gi-G-protein-coupled receptors (Figure 2). The niacin receptor HM74a is coupled to Gi [49] and is activated by the ketone body (D)-β-hydroxybutyrate (see Table 1), an endogenous ligand generated by β-oxidation of fatty acids [50]. Upon fasting, (D)-β-hydroxybutyrate can reach a plasma concentration close to the EC_{50} value for HM74a [50]. Therefore, it plays a key role in mediating

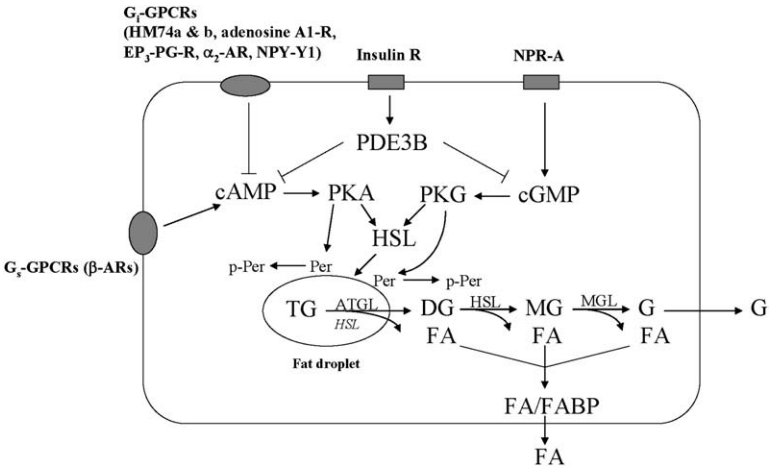
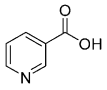
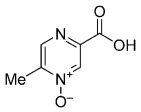
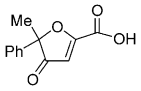
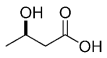
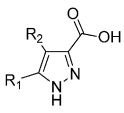
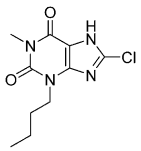
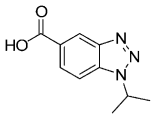


Figure 2. The Lipolysis Pathway in Adipose Tissue

Extracellular signals are mediated by cell surface GPCRs coupled to either Gs or Gi to regulate the intracellular cAMP level and PKA activation. In addition, NPR-A is a non-GPCR cell surface receptor that upon activation increases the intracellular cGMP level and activates PKG. When the intracellular cAMP or cGMP level is increased, the cytosolic HSL is translocated to the surface of the fat droplet, and, in the meantime, phosphorylated perilipin A is displaced to allow HSL access to the core lipids. ATGL is located on the fat droplet, and its expression is upregulated by fasting, glucocorticoids, and other extracellular signals [16, 17]. The FFAs released from lipolysis are bound to FABP and transported out of the cell. FABP, fatty acid binding protein; NPR-A, natriuretic peptide receptor-A; EP3-PG-R, EP3 prostaglandin receptor; NPY-Y1, neuropeptide-Y1 receptor; Per, perilipin A; p-Per, phosphorylated perilipin A; G, glycerol.

Table 1. Structures of Representative HM74a or HM74b Agonists

Compound	Structure	Reference
Niacin (HM74a selective)		[71, 72, 73]
Acipimox (HM74a selective)		[71, 72, 73]
Acifran (nonselective)		[72]
(D) β-hydroxybutyrate (HM74a selective)		[50]
1–3 (HM74a active; no HM74b data)	 **1**, R_1= nBu, R_2 = H **2**, R_1, R_2 =-C_3H_6- **3**, R_1= nPr, R_2 = H	[78]
4 (HM74a selective)		[80]
5 (HM74b selective)		[81]

a negative feedback mechanism to suppress lipolysis. Lipolysis is also regulated by other extracellular factors, such as growth hormone [45], glucocorticoids [51], adipokines [52], and cytokines [53, 54]. Some of the underlying mechanisms are not well understood. Natriuretic peptides exert their lipolytic effect via a non-GPCR receptor that, upon activation, increases the intracellular cGMP level [55] (Figure 2).

Under normal conditions, both the basal and catecholamine-stimulated lipolysis rates per cell are positively correlated with fat cell size [56], which is at least partly attributed to a shift in the balance between β-ARs and α_2-AR [56]. In obesity, the average fat cell volume is increased and the lipolytic rate per cell is elevated [19, 20, 57, 58]. However, when normalized by cell volume or tissue weight, the adipose tissue lipolysis rate remains unchanged in obese subjects compared with normal lean controls [19, 20, 57, 58]. With the higher fat mass in obese subjects, the net result is significantly increased lipolytic activity in the body, resulting in the elevated glycerol levels in both plasma and adipose tissue [58]. The lipolysis in obese subjects also exhibits reduced capacity for catecholamine induction or insulin suppression [19, 20, 57]. Impaired catecholamine-induced lipolysis has been observed in obese children and adults [59, 60], which could be attributed to the reduced expression of HSL and β_2-AR and increased α_2-AR expression in adipose tissue [57, 61, 62]. In addition to fat cell size, fat distribution and regional differences in lipolysis are important in delineating obesity and insulin resistance. Upper body obesity has a greater basal lipolytic rate and reduced catecholamine response compared with lower body obesity [63]. Indeed, the lower body fat has a lower blood flow or lipolysis rate [64] and is associated with favorable glucose and lipid levels [65]. The omental adipose tissue releases more FFAs than subcutaneous fat [66], which promotes FFA delivery through the portal vein to liver and augments hepatic glucose output. This "portal hypothesis" is supported by molecular alterations in the visceral adipose tissue of fat-fed dogs [67]. If this is the main link between central obesity and increased hepatic glucose output, suppression of lipolysis by insulin could play an important role in its inhibitory effect on hepatic gluconeogenesis [68].

The Development of Small-Molecule Modulators for Lipolysis

Since FFAs and glycerol released by lipolysis augment hepatic gluconeogenesis, inhibition of lipolysis could suppress endogenous glucose production. This can be achieved without changing plasma insulin [69]. Based on the findings with niacin and its analogs, it is evident that antilipolytic drugs improve plasma lipoprotein profiles. One of the primary molecular defects in type 2 diabetes and subjects with insulin resistance is the accumulation of intracellular lipid [70]. The subsequent elevation of lipid-derived fatty acid metabolites activates a serine/threonine kinase cascade that ultimately leads to the attenuation of insulin signaling [70]. If elevated plasma FFA level is the primary cause of intracellular lipid accumulation [70], antilipolytic agents are expected to restore insulin signaling. Thus, antilipolytic drugs could improve hepatic and peripheral insulin sensitivity.

Niacin Receptor Agonists

The high-affinity niacin receptor HM74a is a Gi-G-protein-coupled receptor (Gi-GPCR), which upon activation leads to a reduced intracellular cAMP level and subsequent inhibition of HSL [49, 71–74] (Figure 2). HM74a is predominantly expressed in adipose, lung, and spleen [71–73]. A low-affinity receptor HM74b is also coupled to Gi with a similar expression profile, but it is not responsible for the pharmacologic effects of niacin [72, 74]. The role of HM74a in the flushing side effect of niacin is not clear, but its involvement is possible because of its expression in immune cells [75–77].

Despite the unclear role of HM74a in the flushing side effect, small-molecule HM74a agonists have been under investigation (Table 1). One hypothesis is that HM74a causes flushing when activated in skin. Since there is a very low level of HM74a in skin relative to that in adipose tissue, partial agonists are not expected to activate HM74a in skin but to be fully functional in adipose [78].

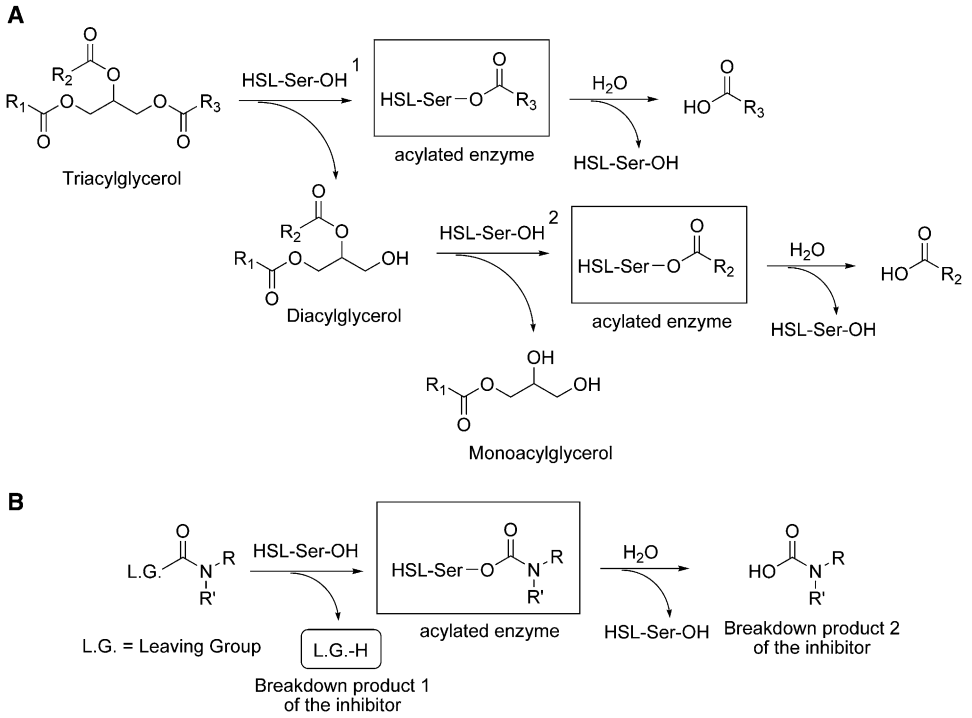


Figure 3. Mechanisms of Catalysis and Pseudosubstrate Inhibition for HSL

(A) Catalysis with TG and DG as substrates. HSL is primarily a DG lipase with significant TG lipase activity. Both reactions are illustrated in the schema.

(B) Proposed mechanism for enzyme inhibition by pseudosubstrates. The pseudosubstrate inhibitor undergoes a nucleophilic attack by the invariant serine at the active site of HSL, forming breakdown product 1 and the acylated enzyme. The subsequent displacement by a water molecule of the fatty acyl chain in the acylated enzyme intermediate results in the release of breakdown product 2 and the reactivated enzyme.

Using this strategy, van Herk and coworkers synthesized a series of pyrazole-3-carboxylic acids based on several known compounds with potent hypolipidemic activities (Table 1, **1–3**) [78, 79]. The maximum activation threshold by these compounds is less than the full activation potential of HM74a, suggesting that they are partial agonists [78]. If partial agonists could render tissue selectivity, the skin flushing could be eliminated with this approach. But experimental data supporting this notion are not available. In contrast to the strategy employed by van Herk and coworkers, Pinto and colleagues took an empirical approach to select xanthine HM74a agonists with low potential for flushing (Table 1, **4**) [80]. To monitor prostaglandin release, they measured the ear temperature of anaesthetized guinea pigs post dosing [80]. The mean increase in ear temperature over time was used to assess a compound's potential to stimulate prostaglandin release. At an identical dose, a xanthine analog induced a much lower temperature increase than niacin [80]. Assuming that this compound is at least as efficacious as niacin at the same dose, this model might be useful in selecting compounds with a low potential of causing skin flushing. Recently, it was suggested that the flushing side effect could be mediated by HM74a, and therefore, HM74b agonism may be a good strategy to achieve antilipolytic effect without causing flushing (Table 1, **5**) [81].

HSL Inhibitors

HSL deficiency in mice improved the lipoprotein profile and inhibited obesity [82, 83], suggesting that HSL-selective inhibitors may be a viable antilipolytic strategy. Due to conserved structural features [84], lipases generally have low substrate specificity. Their hydrolytic potential even extends to phospholipids and organic compounds with an ester bond [85]. However, HSL is an exception in that it is not closely related to other mammalian lipases by sequence comparison, although it contains certain lipase-like segments [86, 87]. Structural modeling based on a bacterial HSL homology revealed its unique structure, suitable for inhibitor selectivity [88].

Catalysis by HSL involves the nucleophilic attack of a serine to the ester carbonyl in the substrate, forming a covalent intermediate with the enzyme. The fatty acyl chain is then displaced by a water molecule to release the FFA (Figure 3A). Many of the reported HSL inhibitors work through this mechanism by acting as pseudosubstrates (Figure 3B). This type of pseudosubstrate inhibition is also widely observed among esterase and protease inhibitors with varying reactivation half-lives [89, 90]. A structure-activity relationship (SAR) may exist for the reactivation half-life, as this has been observed with acetylcholine esterase (AChE) inhibitors [91]. Other HSL inhibitors may also act as pseudosubstrates in a similar mechanism [92–94] (Figure 4). Due to the pseudosubstrate nature, the IC_{50} values of these inhibitors are time dependent. It is important to have ex vivo assays to fully assess a compound's potency.

Although reduced plasma FFAs and glucose have been demonstrated in diabetic rats treated with a selective HSL inhibitor [95], the propensity for nonspecific

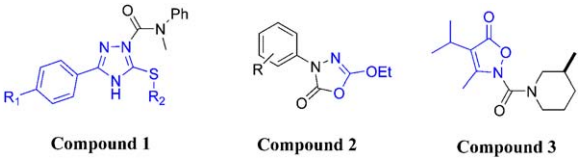


Figure 4. Representative HSL Inhibitors

Compounds **1** [90], **2** [92, 93], and **3** [94, 95] are all potential pseudosubstrate inhibitors. The leaving group (L.G.) for each compound is in blue (also see Figure 3B).

covalent binding is a concern. In addition, the inactivation and reactivation of the enzyme are accompanied with the breakdown of the inhibitor, resulting in the formation of a leaving group and a hydrolyzed fragment, respectively (Figure 3B). Safety assessment of both breakdown products is important. A second potential concern about HSL inhibition is the possible accumulation of intracellular DG, which has been implicated in the activation of a serine/threonine kinase cascade leading to attenuated insulin signaling.

β-Adrenergic Receptors

In contrast to the antilipolytic approach with HM74a agonists and HSL inhibitors, increasing brown adipose tissue (BAT) thermogenesis with β_3-AR agonists has been under investigation for the treatment of obesity. This approach is counterintuitive because β_3-AR agonists stimulate lipolysis, which results in increased FFA release causing insulin resistance and/or dyslipidemia. However, rodent models of obesity and diabetes treated with selective β_3-AR agonists led to marked weight loss and antidiabetic effects [96]. This could be attributed to increased FFA oxidation in thermogenically more active BAT, resulting in reduced obesity and plasma FFAs [96]. But adult humans have very little BAT [97], making this approach questionable. In addition, the antilipolytic α_2-AR level relative to β_3-AR is higher in humans than rodents [98] and further elevated in obese subjects [99], making activating lipolysis via β_3-AR agonism more difficult. In light of these uncertainties, the observed animal efficacy with β_3-AR agonists may not be clinically relevant. However, genetic and polymorphic evidence from human studies suggest a role of β_3-AR in insulin resistance and obesity [100–103], lending some support to the existing β_3-AR agonist efforts [104, 105]. One potential underlying mechanism for these observations is that β_3-AR agonists activate AMP-activated protein kinase (AMPK) [106, 107]. Examples of β_3-AR agonists that have advanced to clinical trials include Rafabegron (AJ-9677) [108] and L-796568 [109, 110] (Figure 5A). In a single-dose study, L-796568 increased energy expenditure, plasma glycerol, and FFAs in obese subjects [109]. In a 28 day study, L-796568 significantly reduced body weight and plasma triglyceride, but no significant increase in energy expenditure or improvement in glucose tolerance was observed [110]. The treated group exhibited an increase in plasma FFAs after the initial dose, but by day 9 the effect diminished, suggesting possible receptor desensitization [110]. Despite signs of efficacy in these clinical studies, inconsistent findings and receptor desensitization are major concerns with this approach.

A1 Receptor Agonists

In addition to HM74a agonists and HSL inhibitors, A1 adenosine receptor agonists are antilipolytic by decreasing the intracellular cAMP level (Figure 2). However, sustained activation of A1 adenosine receptor leads to receptor desensitization [111]. Recent findings indicate that the maximal functional effect mediated by A1 adenosine receptor can be achieved by less than 1% receptor occupancy by a ligand, whereas far greater receptor occupancy is required to cause receptor desensitization [112]. It would appear that careful dose titration may cause minimal or no receptor desensitization while maintaining antilipolytic effects [112]. A second concern about A1 adenosine receptor is its expression in cardiac tissue [113], where its activation could

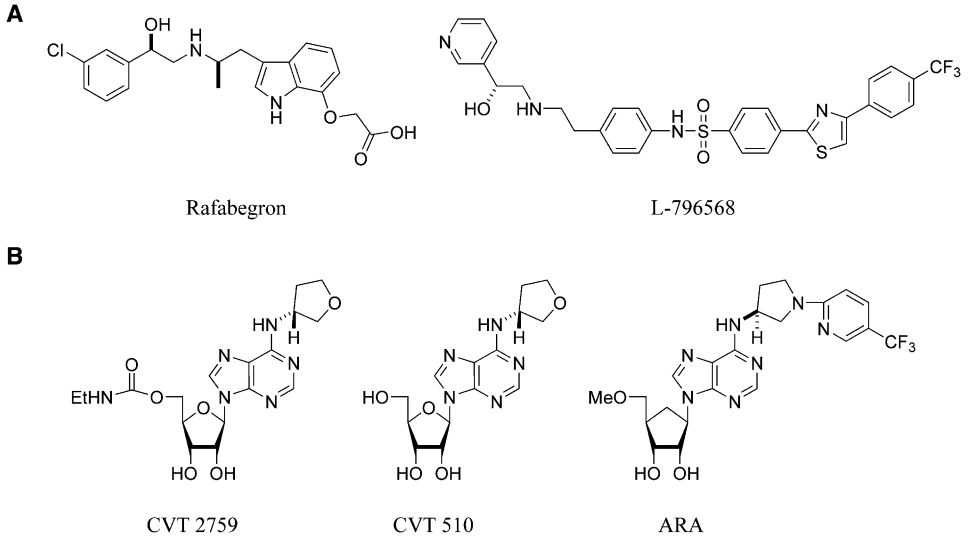


Figure 5. Examples of β_3-AR Agonists and A1 Adenosine Receptor Agonists

(A) Examples of β_3-AR agonists that have been tested in clinical studies, Rafabegron [108] and L-796568 [109, 110].

(B) Examples of A1 adenosine receptor agonists, CVT-2759 [112], CVT-510 [114], and ARA [115].

slow the heart rate. However, since the heart receptor level is >10-fold less than that in the adipocytes [113], a partial agonist at the appropriate dose may not change the heart rate, but still be fully active in adipose with antilipolytic effects. This was demonstrated in a study with A1 partial agonist CVT-2759 [112]. Separate studies with CVT-510 and ARA, respectively, suggest the potential cardiac side effect can also be resolved by careful dose titration [114, 115]. This concept is consistent with the results in a clinical study with CVT-510 [116]. Representative A1 agonists are shown in Figure 5B.

New Targets for Small-Molecule Modulators of Lipolysis

With the mixed clinical results of β_3-AR agonists, more drug discovery efforts are expected to focus on antilipolytic therapeutics. Since the newly identified ATGL is involved in the first step of TG hydrolysis (Figure 2), selective inhibitors targeting this step are likely promising drugs with no risk of DG accumulation. However, ATGL knockout mice have accumulated lipids in the heart, raising safety concerns about this approach [117]. In addition to niacin receptor HM74a and A1 adenosine receptor, other GPCRs might be targeted. EP3 prostaglandin receptor (EP3-PG-R) and neuropeptide-Y1 receptor [118, 119] downregulate the intracellular cAMP level (Figure 2) and therefore could be potential targets for antilipolytic agents. Moreover, atrial natriuretic peptide (ANP) has a potent lipolytic effect in abdominal subcutaneous adipose tissue of healthy subjects [120]. This effect is mediated by natriuretic peptide receptor A (NPR-A) (Figure 2), which upon activation upregulates intracellular cGMP level [55]. The serum ANP level is correlated with the venous glycerol concentration in humans [121], suggesting that it may play a role in lipolysis. Since NPR-A is expressed in human adipose tissue, ANP antagonists are potential antilipolytic compounds for the treatment of insulin resistance and dyslipidemia [122]. Natriuretic receptors may only be expressed in primate adipose tissues but not in rodent adipose tissues. Therefore, the efficacy of ANP antagonists can not be assessed in rodent systems.

Key Issues and Future Directions

The concept of using stimulators of lipolysis for the treatment of obesity has been tested in clinical trials with β_3-AR agonists. Since the rationale is largely based on findings in rodent models, and given the large differences in β_3-AR agonism between human and rodents, the potential of these molecules as drugs is debatable and remains to be further validated in clinic. The antilipolytic effects of niacin and other niacin analogs are beneficial, but the side effects need to be minimized or eliminated. The next generation of molecules must meet higher safety standards, since they will be used to treat chronic conditions such as insulin resistance and obesity. Therefore, targets that are expressed specifically in adipose tissue are preferred, and compounds that act on these targets have to be selective. Since the lipolytic pathway is highly regulated via signaling, receptor desensitization is an issue that needs to be considered when selecting targets and lead compounds. With the accumulating knowledge of the lipolytic pathway, especially the clinical experience with a number of compounds, continued efforts in this area will likely lead to new chemical entities for the treatment of insulin resistance, dyslipidemia, and obesity.

Acknowledgments

The authors are employed researchers in a biopharmaceutical company.

References

1. Reaven, G.M., Hollenbeck, C., Jeng, C.Y., Wu, M.S., and Chen, Y.D. (1988). Measurement of plasma glucose, free fatty acid, lactate, and insulin for 24 h in patients with NIDDM. Diabetes *37*, 1020–1024.
2. Pankow, J.S., Duncan, B.B., Schmidt, M.I., Ballantyne, C.M., Couper, D.J., Hoogeveen, R.C., and Golden, S.H. (2004). Atherosclerosis Risk in Communities Study. Fasting plasma free fatty acids and risk of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes Care *27*, 77–82.
3. Paolisso, G., Tataranni, P.A., Foley, J.E., Bogardus, C., Howard, B.V., and Ravussin, E. (1995). A high concentration of fasting plasma non-esterified fatty acids is a risk factor for the development of NIDDM. Diabetologia *38*, 1213–1217.
4. Havel, R.J. (1961). Conversion of plasma free fatty acids into triglycerides of plasma lipoprotein fractions in man. Metabolism *10*, 1031–1036.
5. Ferrannini, E., Barrett, E.J., Bevilacqua, S., and DeFronzo, R.A. (1983). Effect of fatty acids on glucose production and utilization in man. J. Clin. Invest. *72*, 1737–1747.
6. Iozzo, P., Lautamaki, R., Geisler, F., Virtanen, K.A., Oikonen, V., Haaparanta, M., Yki-Jarvinen, H., Ferrannini, E., Knuuti, J., and Nuutila, P. (2004). Non-esterified fatty acids impair insulin-mediated glucose uptake and disposition in the liver. Diabetologia *47*, 1149–1156.
7. Chen, X., Iqbal, N., and Boden, G. (1999). The effects of free fatty acids on gluconeogenesis and glycogenolysis in normal subjects. J. Clin. Invest. *103*, 365–372.
8. Roden, M., Krssak, M., Stingl, H., Gruber, S., Hofer, A., Furnsinn, C., Moser, E., and Waldhausl, W. (1999). Rapid impairment of skeletal muscle glucose transport/phosphorylation by free fatty acids in humans. Diabetes *48*, 358–364.
9. Homko, C.J., Cheung, P., and Boden, G. (2003). Effects of free fatty acids on glucose uptake and utilization in healthy women. Diabetes *52*, 487–491.
10. de Jongh, R.T., Serne, E.H., Ijzerman, R.G., de Vries, G., and Stehouwer, C.D. (2004). Free fatty acid levels modulate microvascular function: relevance for obesity-associated insulin resistance, hypertension, and microangiopathy. Diabetes *53*, 2873–2882.
11. Dubois, M., Kerr-Conte, J., Gmyr, V., Bouckenooghe, T., Muharram, G., D'Herbomez, M., Martin-Ponthieu, A., Vantyghem, M.C., Vandewalle, B., and Pattou, F. (2004). Non-esterified fatty acids are deleterious for human pancreatic islet function at physiological glucose concentration. Diabetologia *47*, 463–469.
12. Boden, G., and Shulman, G.I. (2002). Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. Eur. J. Clin. Invest. *32* (*Suppl. 3*), 14–23.
13. Yeaman, S.J. (2000). Hormone-sensitive lipase—new roles for an old enzyme. Biochem. J. *379*, 11–22.
14. Kershaw, E.E., Hamm, J.K., Verhagen, L.A., Peroni, O., Katic, M., and Flier, J.S. (2006). Adipose triglyceride lipase: function, regulation by insulin, and comparison with adiponutrin. Diabetes *55*, 148–157.
15. Zimmermann, R., Strauss, J.G., Haemmerle, G., Schoiswohl, G., Birner-Gruenberger, R., Riederer, M., Lass, A., Neuberger, G., Eisenhaber, F., Hermetter, A., et al. (2004). Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science *306*, 1383–1386.
16. Villena, J.A., Roy, S., Sarkadi-Nagy, E., Kim, K.H., and Sul, H.S. (2004). Desnutrin, an adipocyte gene encoding a novel patatin domain-containing protein, is induced by fasting and

glucocorticoids: ectopic expression of desnutrin increases triglyceride hydrolysis. J. Biol. Chem. *279*, 47066–47075.

17. Jenkins, C.M., Mancuso, D.J., Yan, W., Sims, H.F., Gibson, B., and Gross, R.W. (2004). Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A_2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. J. Biol. Chem. *279*, 48968–48975.
18. Hales, C.N., Luzio, J.P., and Siddle, K. (1978). Hormonal control of adipose-tissue lipolysis. Biochem. Soc. Symp. *43*, 97–135.
19. Skowronski, R., Hollenbeck, C.B., Varasteh, B.B., Chen, Y.D., and Reaven, G.M. (1991). Regulation of non-esterified fatty acid and glycerol concentration by insulin in normal individuals and patients with type 2 diabetes. Diabet. Med. *8*, 330–333.
20. Campbell, P.J., Carlson, M.G., and Nurjhan, N. (1994). Fat metabolism in human obesity. Am. J. Physiol. *266*, E600–E605.
21. Nurjhan, N., Consoli, A., and Gerich, J. (1992). Increased lipolysis and its consequences on gluconeogenesis in non-insulin-dependent diabetes mellitus. J. Clin. Invest. *89*, 169–175.
22. Carlson, L.A. (2005). Nicotinic acid: the broad-spectrum lipid drug. A 50th anniversary review. J. Intern. Med. *258*, 94–114.
23. Bajaj, M., Suraamornkul, S., Kashyap, S., Cusi, K., Mandarino, L., and DeFronzo, R.A. (2004). Sustained reduction in plasma free fatty acid concentration improves insulin action without altering plasma adipocytokine levels in subjects with strong family history of type 2 diabetes. J. Clin. Endocrinol. Metab. *89*, 4649–4655.
24. Vaag, A., Skott, P., Damsbo, P., Gall, M.A., Richter, E.A., and Beck-Nielsen, H. (1991). Effect of the antilipolytic nicotinic acid analogue acipimox on whole-body and skeletal muscle glucose metabolism in patients with non-insulin-dependent diabetes mellitus. J. Clin. Invest. *88*, 1282–1290.
25. Boden, G., Chen, X., and Iqbal, N. (1998). Acute lowering of plasma fatty acids lowers basal insulin secretion in diabetic and nondiabetic subjects. Diabetes *47*, 1609–1612.
26. Santomauro, A.T., Boden, G., Silva, M.E., Rocha, D.M., Santos, R.F., Ursich, M.J., Strassmann, P.G., and Wajchenberg, B.L. (1999). Overnight lowering of free fatty acids with Acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects. Diabetes *48*, 1836–1841.
27. Stern, R.H., Spence, J.D., Freeman, D.J., and Parbtani, A. (1991). Tolerance to nicotinic acid flushing. Clin. Pharmacol. Ther. *50*, 66–70.
28. Morrow, J.D., Awad, J.A., Oates, J.A., and Roberts, L.J., II. (1992). Identification of skin as a major site of prostaglandin D_2 release following oral administration of niacin in humans. J. Invest. Dermatol. *98*, 812–815.
29. Dalton, T.A., and Berry, R.S. (1992). Hepatotoxicity associated with sustained-release niacin. Am. J. Med. *93*, 102–104.
30. Kahn, S.E., Beard, J.C., Schwartz, M.W., Ward, W.K., Ding, H.L., Bergman, R.N., Taborsky, G.J., Jr., and Porte, D., Jr. (1989). Increased beta-cell secretory capacity as mechanism for islet adaptation to nicotinic acid-induced insulin resistance. Diabetes *38*, 562–568.
31. Garg, A., and Grundy, S.M. (1990). Nicotinic acid as therapy for dyslipidemia in non-insulin-dependent diabetes mellitus. JAMA *264*, 723–726.
32. Soni, K.G., Lehner, R., Metalnikov, P., O'Donnell, P., Semache, M., Gao, W., Ashman, K., Pshezhetsky, A.V., and Mitchell, G.A. (2004). Carboxylesterase 3 (EC 3.1.1.1) is a major adipocyte lipase. J. Biol. Chem. *279*, 40683–40689.
33. Schoenborn, V., Heid, I.M., Vollmert, C., Lingenhel, A., Adams, T.D., Hopkins, P.N., Illig, T., Zimmermann, R., Zechner, R., Hunt, S.C., et al. (2006). The ATGL gene is associated with free fatty acids, triglycerides, and type 2 diabetes. Diabetes *55*, 1270–1275.
34. Osuga, J., Ishibashi, S., Oka, T., Yagyu, H., Tozawa, R., Fujimoto, A., Shionoiri, F., Yahagi, N., Kraemer, F.B., Tsutsumi, O., et al. (2000). Targeted disruption of hormone-sensitive lipase results in male sterility and adipocyte hypertrophy, but not in obesity. Proc. Natl. Acad. Sci. USA *97*, 787–792.
35. Wang, S.P., Laurin, N., Himms-Hagen, J., Rudnicki, M.A., Levy, E., Robert, M.F., Pan, L., Oligny, L., and Mitchell, G.A. (2001). The adipose tissue phenotype of hormone-sensitive lipase deficiency in mice. Obes. Res. *9*, 119–128.
36. Haemmerle, G., Zimmermann, R., Hayn, M., Theussl, C., Waeg, G., Wagner, E., Sattler, W., Magin, T.M., Wagner, E.F., and Zechner, R. (2002). Hormone-sensitive lipase deficiency in mice causes diglyceride accumulation in adipose tissue, muscle, and testis. J. Biol. Chem. *277*, 4806–4815.
37. Sztalryd, C., Xu, G., Dorward, H., Tansey, J.T., Contreras, J.A., Kimmel, A.R., and Londos, C. (2003). Perilipin A is essential for the translocation of hormone-sensitive lipase during lipolytic activation. J. Cell Biol. *161*, 1093–1103, Erratum: J. Cell Biol. (2003). *162*, 353.
38. Martinez-Botas, J., Anderson, J.B., Tessier, D., Lapillonne, A., Chang, B.H., Quast, M.J., Gorenstein, D., Chen, K.H., and Chan, L. (2000). Absence of perilipin results in leanness and reverses obesity in Lepr(db/db) mice. Nat. Genet. *26*, 474–479.
39. Mottagui-Tabar, S., Ryden, M., Lofgren, P., Faulds, G., Hoffstedt, J., Brookes, A.J., Andersson, I., and Arner, P. (2003). Evidence for an important role of perilipin in the regulation of human adipocyte lipolysis. Diabetologia *46*, 789–797.
40. Wang, Y., Sullivan, S., Trujillo, M., Lee, M.J., Schneider, S.H., Brolin, R.E., Kang, Y.H., Werber, Y., Greenberg, A.S., and Fried, S.K. (2003). Perilipin expression in human adipose tissues: effects of severe obesity, gender, and depot. Obes. Res. *11*, 930–936.
41. Jenkins-Kruchten, A.E., Bennaars-Eiden, A., Ross, J.R., Shen, W.J., Kraemer, F.B., and Bernlohr, D.A. (2003). Fatty acid-binding protein-hormone-sensitive lipase interaction. Fatty acid dependence on binding. J. Biol. Chem. *278*, 47636–47643.
42. Smith, A.J., Sanders, M.A., Thompson, B.R., Londos, C., Kraemer, F.B., and Bernlohr, D.A. (2004). Physical association between the adipocyte fatty acid-binding protein and hormone-sensitive lipase: a fluorescence resonance energy transfer analysis. J. Biol. Chem. *279*, 52399–52405.
43. Hertzel, A.V., Smith, L.A., Berg, A.H., Cline, G.W., Shulman, G.I., Scherer, P.E., and Bernlohr, D.A. (2006). Lipid metabolism and adipokine levels in fatty acid-binding protein null and transgenic mice. Am. J. Physiol. Endocrinol. Metab. *290*, E814–E823.
44. Hotamisligil, G.S., Johnson, R.S., Distel, R.J., Ellis, R., Papaioannou, V.E., and Spiegelman, B.M. (1996). Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. Science *274*, 1377–1379.
45. Lafontan, M., and Berlan, M. (1993). Fat cell adrenergic receptors and the control of white and brown fat cell function. J. Lipid Res. *34*, 1057–1091.
46. Degerman, E., Smith, C.J., Tornqvist, H., Vasta, V., Belfrage, P., and Manganiello, V.C. (1990). Evidence that insulin and isoprenaline activate the cGMP-inhibited low-Km cAMP phosphodiesterase in rat fat cells by phosphorylation. Proc. Natl. Acad. Sci. USA *87*, 533–537.
47. Kitamura, T., Kitamura, Y., Kuroda, S., Hino, Y., Ando, M., Kotani, K., Konishi, H., Matsuzaki, H., Kikkawa, U., Ogawa, W., et al. (1999). Insulin-induced phosphorylation and activation of cyclic nucleotide phosphodiesterase 3B by the serine-threonine kinase Akt. Mol. Cell. Biol. *19*, 6286–6296.
48. Snyder, P.B., Esselstyn, J.M., Loughney, K., Wolda, S.L., and Florio, V.A. (2005). The role of cyclic nucleotide phosphodiesterases in the regulation of adipocyte lipolysis. J. Lipid Res. *46*, 494–503.
49. Zhang, Y., Schmidt, R.J., Foxworthy, P., Emkey, R., Oler, J.K., Large, T.H., Wang, H., Su, E.W., Mosior, M.K., Eacho, P.I., et al. (2005). Niacin mediates lipolysis in adipose tissue through its G-protein coupled receptor HM74a. Biochem. Biophys. Res. Commun. *334*, 729–732.
50. Taggart, A.K., Kero, J., Gan, X., Cai, T.Q., Cheng, K., Ippolito, M., Ren, N., Kaplan, R., Wu, K., Wu, T.J., et al. (2005). (D)-beta-Hydroxybutyrate inhibits adipocyte lipolysis via the nicotinic acid receptor PUMA-G. J. Biol. Chem. *280*, 26649–26652.
51. Divertie, G.D., Jensen, M.D., and Miles, J.M. (1991). Stimulation of lipolysis in humans by physiological hypercortisolemia. Diabetes *40*, 1228–1232.

52. Fruhbeck, G., Gomez-Ambrosi, J., and Salvador, J. (2001). Leptin-induced lipolysis opposes the tonic inhibition of endogenous adenosine in white adipocytes. FASEB J. *15*, 333–340.
53. Ryden, M., Arvidsson, E., Blomqvist, L., Perbeck, L., Dicker, A., and Arner, P. (2004). Targets for TNFα-induced lipolysis in human adipocytes. Biochem. Biophys. Res. Commun. *318*, 168–175.
54. Lyngso, D., Simonsen, L., and Bulow, J. (2002). Interleukin-6 production in human subcutaneous abdominal adipose tissue: the effect of exercise. J. Physiol. *543*, 373–378.
55. Moro, C., Galitzky, J., Sengenes, C., Crampes, F., Lafontan, M., and Berlan, M. (2003). Functional and pharmacological characterization of the natriuretic peptide-dependent lipolytic pathway in human fat cells. J. Pharmacol. Exp. Ther. *308*, 984–992.
56. Engfeldt, P., and Arner, P. (1988). Lipolysis in human adipocytes, effects of cell size, age and of regional differences. Horm. Metab. Res. Suppl. *19*, 26–29.
57. Large, V., Reynisdottir, S., Langin, D., Fredby, K., Klannemark, M., Holm, C., and Arner, P. (1999). Decreased expression and function of adipocyte hormone-sensitive lipase in subcutaneous fat cells of obese subjects. J. Lipid Res. *40*, 2059–2066.
58. Bolinder, J., Kerckhoffs, D.A., Moberg, E., Hagstrom-Toft, E., and Arner, P. (2000). Rates of skeletal muscle and adipose tissue glycerol release in nonobese and obese subjects. Diabetes *49*, 797–802.
59. Bougneres, P., Stunff, C.L., Pecqueur, C., Pinglier, E., Adnot, P., and Ricquier, D. (1997). In vivo resistance of lipolysis to epinephrine. A new feature of childhood onset obesity. J. Clin. Invest. *99*, 2568–2573.
60. Reynisdottir, S., Wahrenberg, H., Carlstrom, K., Rossner, S., and Arner, P. (1994). Catecholamine resistance in fat cells of women with upper-body obesity due to decreased expression of β2-adrenoceptors. Diabetologia *37*, 428–435.
61. Langin, D., Dicker, A., Tavernier, G., Hoffstedt, J., Mairal, A., Ryden, M., Arner, E., Sicard, A., Jenkins, C.M., Viguerie, N., et al. (2005). Adipocyte lipases and defect of lipolysis in human obesity. Diabetes *54*, 3190–3197.
62. Mauriege, P., Despres, J.P., Prud'homme, D., Pouliot, M.C., Marcotte, M., Tremblay, A., and Bouchard, C. (1991). Regional variation in adipose tissue lipolysis in lean and obese men. J. Lipid Res. *32*, 1625–1633.
63. Jensen, M.D., Haymond, M.W., Rizza, R.A., Cryer, P.E., and Miles, J.M. (1989). Influence of body fat distribution on free fatty acid metabolism in obesity. J. Clin. Invest. *83*, 1168–1173.
64. Tan, G.D., Goossens, G.H., Humphreys, S.M., Vidal, H., and Karpe, F. (2004). Upper and lower body adipose tissue function: a direct comparison of fat mobilization in humans. Obes. Res. *12*, 114–118.
65. Snijder, M.B., Visser, M., Dekker, J.M., Goodpaster, B.H., Harris, T.B., Kritchevsky, S.B., De Rekeneire, N., Kanaya, A.M., Newman, A.B., Tylavsky, F.A., and Seidell, J.C. (2005). Low subcutaneous thigh fat is a risk factor for unfavourable glucose and lipid levels, independently of high abdominal fat. The Health ABC Study. Diabetologia *48*, 301–308.
66. Richelsen, B., Pedersen, S.B., Moller-Pedersen, T., and Bak, J.F. (1991). Regional differences in triglyceride breakdown in human adipose tissue: effects of catecholamines, insulin, and prostaglandin E_2. Metabolism *40*, 990–996.
67. Kabir, M., Catalano, K.J., Ananthnarayan, S., Kim, S.P., Van Citters, G.W., Dea, M.K., and Bergman, R.N. (2005). Molecular evidence supporting the portal theory: a causative link between visceral adiposity and hepatic insulin resistance. Am. J. Physiol. Endocrinol. Metab. *288*, E454–E461.
68. Bergman, R.N. (2000). Non-esterified fatty acids and the liver: why is insulin secreted into the portal vein? Diabetologia *43*, 946–952.
69. Mittelman, S.D., and Bergman, R.N. (2000). Inhibition of lipolysis causes suppression of endogenous glucose production independent of changes in insulin. Am. J. Physiol. Endocrinol. Metab. *279*, E630–E637.
70. Petersen, K.F., and Shulman, G.I. (2006). New insights into the pathogenesis of insulin resistance in humans using magnetic resonance spectroscopy. Obesity (Silver Spring) *14* (*Suppl. 1*), 34S–40S.
71. Lorenzen, A., Stannek, C., Lang, H., Andrianov, V., Kalvinsh, I., and Schwabe, U. (2001). Characterization of a G protein-coupled receptor for nicotinic acid. Mol. Pharmacol. *59*, 349–357.
72. Wise, A., Foord, S.M., Fraser, N.J., Barnes, A.A., Elshourbagy, N., Eilert, M., Ignar, D.M., Murdock, P.R., Steplewski, K., Green, A., et al. (2003). Molecular identification of high and low affinity receptors for nicotinic acid. J. Biol. Chem. *278*, 9869–9874.
73. Soga, T., Kamohara, M., Takasaki, J., Matsumoto, S., Saito, T., Ohishi, T., Hiyama, H., Matsuo, A., Matsushime, H., and Furuichi, K. (2003). Molecular identification of nicotinic acid receptor. Biochem. Biophys. Res. Commun. *303*, 364–369.
74. Tunaru, S., Kero, J., Schaub, A., Wufka, C., Blaukat, A., Pfeffer, K., and Offermanns, S. (2003). PUMA-G and HM74 are receptors for nicotinic acid and mediate its anti-lipolytic effect. Nat. Med. *9*, 352–355.
75. Schaub, A., Futterer, A., and Pfeffer, K. (2001). PUMA-G, an IFN-gamma-inducible gene in macrophages is a novel member of the seven transmembrane spanning receptor superfamily. Eur. J. Immunol. *31*, 3714–3725.
76. Pike, N.B., and Wise, A. (2004). Identification of a nicotinic acid receptor: is this the molecular target for the oldest lipid-lowering drug? Curr. Opin. Investig. Drugs *5*, 271–275.
77. Yousefi, S., Cooper, P.R., Mueck, B., Potter, S.L., and Jarai, G. (2000). cDNA representational difference analysis of human neutrophils stimulated by GM-CSF. Biochem. Biophys. Res. Commun. *277*, 401–409.
78. van Herk, T., Brussee, J., van den Nieuwendijk, A.M., van der Klein, P.A., Ijzerman, A.P., Stannek, C., Burmeister, A., and Lorenzen, A. (2003). Pyrazole derivatives as partial agonists for the nicotinic acid receptor. J. Med. Chem. *46*, 3945–3951.
79. Seki, K., Isegawa, J., Fukuda, M., and Ohki, M. (1984). Studies on hypolipidemic agents. II. Synthesis and pharmacological properties of alkylpyrazole derivatives. Chem. Pharm. Bull. (Tokyo) *32*, 1568–1577.
80. Pinto, I.L., Rahman, S.S., and Nicholson, N.H. (2005). Preparation of xanthine derivatives as HM74A agonists. PCT Int. Appl. WO 2005077950.
81. Semple, G., Skinner, P.J., Cherrier, M.C., Webb, P.J., Sage, C.R., Tamura, S.Y., Chen, R., Richman, J.G., and Connolly, D.T. (2006). 1-alkyl-benzotriazole-5-carboxylic acids are highly selective agonists of the human orphan G-protein-coupled receptor GPR109b. J. Med. Chem. *49*, 1227–1230.
82. Haemmerle, G., Zimmermann, R., Strauss, J.G., Kratky, D., Riederer, M., Knipping, G., and Zechner, R. (2002). Hormone-sensitive lipase deficiency in mice changes the plasma lipid profile by affecting the tissue-specific expression pattern of lipoprotein lipase in adipose tissue and muscle. J. Biol. Chem. *277*, 12946–12952.
83. Sekiya, M., Osuga, J., Okazaki, H., Yahagi, N., Harada, K., Shen, W.J., Tamura, Y., Tomita, S., Iizuka, Y., Ohashi, K., et al. (2004). Absence of hormone-sensitive lipase inhibits obesity and adipogenesis in Lep ob/ob mice. J. Biol. Chem. *279*, 15084–15090.
84. Ollis, D.L., Cheah, E., Cygler, M., Dijkstra, B., Frolow, F., Franken, S.M., Harel, M., Remington, S.J., Silman, I., Schrag, J., et al. (1992). The α/β hydrolase fold. Protein Eng. *5*, 197–211.
85. Derewenda, Z.S. (1994). Structure and function of lipases. Adv. Protein Chem. *45*, 1–52.
86. Osterlund, T., Danielsson, B., Degerman, E., Contreras, J.A., Edgren, G., Davis, R.C., Schotz, M.C., and Holm, C. (1996). Domain-structure analysis of recombinant rat hormone-sensitive lipase. Biochem. J. *319*, 411–420.
87. Contreras, J.A., Karlsson, M., Osterlund, T., Laurell, H., Svensson, A., and Holm, C. (1996). Hormone-sensitive lipase is structurally related to acetylcholinesterase, bile salt-stimulated lipase, and several fungal lipases. Building of a three-dimensional model for the catalytic domain of hormone-sensitive lipase. J. Biol. Chem. *271*, 31426–31430.
88. Wei, Y., Contreras, J.A., Sheffield, P., Osterlund, T., Derewenda, U., Kneusel, R.E., Matern, U., Holm, C., and Derewenda, Z.S. (1999). Crystal structure of brefeldin A esterase, a bacterial homolog of the mammalian hormone-sensitive lipase. Nat. Struct. Biol. *6*, 340–345.

89. Perola, E., Cellai, L., Lamba, D., Filocamo, L., and Brufani, M. (1997). Long chain analogs of physostigmine as potential drugs for Alzheimer's disease: new insights into the mechanism of action in the inhibition of acetylcholinesterase. Biochim. Biophys. Acta *1343*, 41–50.
90. Ebdrup, S., Sorensen, L.G., Olsen, O.H., and Jacobsen, P. (2004). Synthesis and structure-activity relationship for a novel class of potent and selective carbamoyl-triazole based inhibitors of hormone sensitive lipase. J. Med. Chem. *47*, 400–410.
91. Rampa, A., Piazzi, L., Belluti, F., Gobbi, S., Bisi, A., Bartolini, M., Andrisano, V., Cavrini, V., Cavalli, A., Recanatini, M., et al. (2001). Acetylcholinesterase inhibitors: SAR and kinetic studies on omega-[N-methyl-N-(3-alkylcarbamoyloxyphenyl)methyl] aminoalkoxyaryl derivatives. J. Med. Chem. *44*, 3810–3820.
92. Petry, S., Schoenafinger, K., Mueller, G., and Baringhaus, K.-H. (2001). Preparation of 3-phenyl-5-alkoxy-1,3,4-oxadiazol-2-ones as hormone-sensitive lipase inhibitors. Ger. Offen. DE 19942354.
93. Schoenafinger, K., Petry, S., Mueller, G., and Baringhaus, K.-H. (2001). Preparation of 3-phenyl-5-alkoxy-1,3,4-oxadiazol-2-ones as inhibitors of hormone sensitive lipase (HSL). Ger. Offen. DE 10010968.
94. Lowe, D.B., Magnuson, S., Qi, N., Campbell, A.-M., Cook, J., Hong, Z., Wang, M., Rodriguez, M., Achebe, F., Kluender, H., et al. (2004). In vitro SAR of (5-(2H)-isoxazolonyl) ureas, potent inhibitors of hormone-sensitive lipase. Bioorg. Med. Chem. Lett. *14*, 3155–3159.
95. Claus, T.H., Lowe, D.B., Liang, Y., Salhanick, A.I., Lubeski, C.K., Yang, L., Lemoine, L., Zhu, J., and Clairmont, K.B. (2005). Specific inhibition of hormone-sensitive lipase improves lipid profile while reducing plasma glucose. J. Pharmacol. Exp. Ther. *315*, 1396–1402.
96. de Souza, C.J., and Burkey, B.F. (2001). β3-adrenoceptor agonists as anti-diabetic and anti-obesity drugs in humans. Cur. Pharm. Des. *7*, 1433–1449.
97. Himms-Hagen, J., and Ricquier, D. (1998). Brown adipose tissue. In Handbook of Obesity, G.A. Bray, C. Bouchard, and W.P.T. James, eds. (New York: M. Dekker), pp. 415–441.
98. Collins, S., Cao, W., and Robidoux, J. (2004). Learning new tricks from old dogs: β-adrenergic receptors teach new lessons on firing up adipose tissue metabolism. Mol. Endocrinol. *18*, 2123–2131.
99. Lafontan, M., Sengenes, C., Galitzky, J., Berlan, M., De Glisezinski, I., Crampes, F., Stich, V., Langin, D., Barbe, P., and Riviere, D. (2000). Recent developments on lipolysis regulation in humans and discovery of a new lipolytic pathway. Int. J. Obes. Relat. Metab. Disord. *24* (*Suppl. 4*), S47–S52.
100. Walston, J., Silver, K., Bogardus, C., Knowler, W.C., Celi, F.S., Austin, S., Manning, B., Strosberg, A.D., Stern, M.P., Raben, N., et al. (1995). Time of onset of non-insulin-dependent diabetes mellitus and genetic variation in the β3-adrenergic-receptor gene. N. Engl. J. Med. *333*, 343–347.
101. Widen, E., Lehto, M., Kanninen, T., Walston, J., Shuldiner, A.R., and Groop, L.C. (1995). Association of a polymorphism in the β3-adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. N. Engl. J. Med. *333*, 348–351.
102. Clement, K., Vaisse, C., Manning, B.S., Basdevant, A., Guy-Grand, B., Ruiz, J., Silver, K.D., Shuldiner, A.R., Froguel, P., and Strosberg, A.D. (1995). Genetic variation in the β3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. N. Engl. J. Med. *333*, 352–354.
103. Yoshida, T., Sakane, N., Umekawa, T., Sakai, M., Takahashi, T., and Kondo, M. (1995). Mutation of β3-adrenergic-receptor gene and response to treatment of obesity. Lancet *346*, 1433–1434.
104. Hu, B., and Jennings, L.J. (2003). Orally bioavailable β3-adrenergic receptor agonists as potential therapeutic agents for obesity ad type-II diabetes. Prog. Med. Chem. *41*, 167–194.
105. Nisoli, E., Tonello, C., and Carruba, M.O. (2003). β3-adrenergic receptor: really relevant targets for anti-obesity drugs? Curr. Med. Chem. Central Nervous System Agents *3*, 257–273.
106. Moule, S.K., and Denton, R.M. (1998). The activation of p38 MAPK by the β-adrenergic agonist isoproterenol in rat epididymal fat cells. FEBS Lett. *439*, 287–290.
107. Yin, W., Mu, J., and Birnbaum, M.J. (2003). Role of AMP-activated protein kinase in cyclic AMP-dependent lipolysis in 3T3-L1 adipocytes. J. Biol. Chem. *278*, 43074–43080.
108. Sugimoto, T., Ogawa, W., Kasuga, M., and Yokoyama, Y. (2005). Chronic effects of AJ-9677 on energy expenditure and energy source utilization in rats. Eur. J. Pharmacol. *519*, 135–145.
109. Van Baak, M.A., Hul, G.B.J., Toubro, S., Astrup, A., Gottesdiener, K.M., DeSmet, M., and Saris, W.H.M. (2002). Acute effect of L-796568, a novel β3-adrenergic receptor agonist, on energy expenditure in obese men. Clin. Pharmacol. Ther. *71*, 272–279.
110. Larsen, T.M., Toubro, S., van Baak, M.A., Gottesdiener, K.M., Larson, P., Saris, W.H.M., and Astrup, A. (2002). Effect of a 28-d treatment with L-796568, a novel β3-adrenergic receptor agonist, on energy expenditure and body composition in obese men. Am. J. Clin. Nutr. *76*, 780–788.
111. Green, A. (1987). Adenosine receptor down-regulation and insulin resistance following prolonged incubation of adipocytes with an A1 adenosine receptor agonist. J. Biol. Chem. *262*, 15702–15707.
112. Liang, H.-X., Belardinelli, L., Ozeck, M.J., and Shryock, J.C. (2002). Tonic activity of the rat adipocyte A1-adenosine receptor. Br. J. Pharmacol. *135*, 1457–1466.
113. Srinivas, M., Shryock, J.C., Dennis, D.M., Baker, S.P., and Belardinelli, L. (1997). Differential A1 adenosine receptor reserve for two actions of adenosine on guinea pig atrial myocytes. Mol. Pharmacol. *52*, 683–691.
114. Fraser, H., Gao, Z., Ozeck, M.J., and Belardinelli, L. (2003). N-[3-(R)-tetrahydrofuranyl]-6-aminopurine riboside, an A1 adenosine receptor agonist, antagonizes catecholamine-induced lipolysis without cardiovascular effects in awake rats. J. Pharmacol. Exp. Ther. *305*, 225–231.
115. Schoelch, C., Kuhlmann, J., Gossel, M., Mueller, G., Neumann-Haefelin, C., Belz, U., Kalisch, J., Biemer-Daub, G., Kramer, W., Juretschke, H.-P., et al. (2004). Characterization of adenosine-A1 receptor-mediated antilipolysis in rats by tissue microdialysis, ^{1}H-spectroscopy, and glucose clamp studies. Diabetes *53*, 1920–1926.
116. Dhalla, A.K., Shryock, J.C., Shreeniwas, R., and Belardinelli, L. (2003). Pharmacology and therapeutic applications of A1 adenosine receptor ligands. Curr. Top. Med. Chem. *3*, 369–385.
117. Haemmerle, G., Lass, A., Zimmermann, R., Gorkiewicz, G., Meyer, C., Rozman, J., Heldmaier, G., Maier, R., Theussl, C., Eder, S., et al. (2006). Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. Science *312*, 734–737.
118. Hatae, N., Sugimoto, Y., and Ichikawa, A. (2002). Prostaglandin receptors: advances in the study of EP3 receptor signaling. J. Biochem. (Tokyo) *131*, 781–784.
119. Valet, P., Berlan, M., Beauville, M., Crampes, F., Montastruc, J.L., and Lafontan, M. (1990). Neuropeptide Y and peptide YY inhibit lipolysis in human and dog fat cells through a pertussis toxin-sensitive G protein. J. Clin. Invest. *85*, 291–295.
120. Sengenes, C., Berlan, M., De Glisezinski, I., Lafontan, M., and Galitzky, J. (2000). Natriuretic peptides: a new lipolytic pathway in human adipocytes. FASEB J. *14*, 1345–1351.
121. Birkenfeld, A.L., Boschmann, M., Moro, C., Adams, F., Heusser, K., Franke, G., Berlan, M., Luft, F.C., Lafontan, M., and Jordan, J. (2005). Lipid mobilization with physiological atrial natriuretic peptide concentrations in humans. J. Clin. Endocrinol. Metab. *90*, 3622–3628.
122. Lafontan, M., Moro, C., Sengenes, C., Galitzky, J., Crampes, F., and Berlan, M. (2005). An unsuspected metabolic role for atrial natriuretic peptides: the control of lipolysis, lipid mobilization, and systemic nonesterified fatty acids levels in humans. Arterioscler. Thromb. Vasc. Biol. *25*, 2032–2042.