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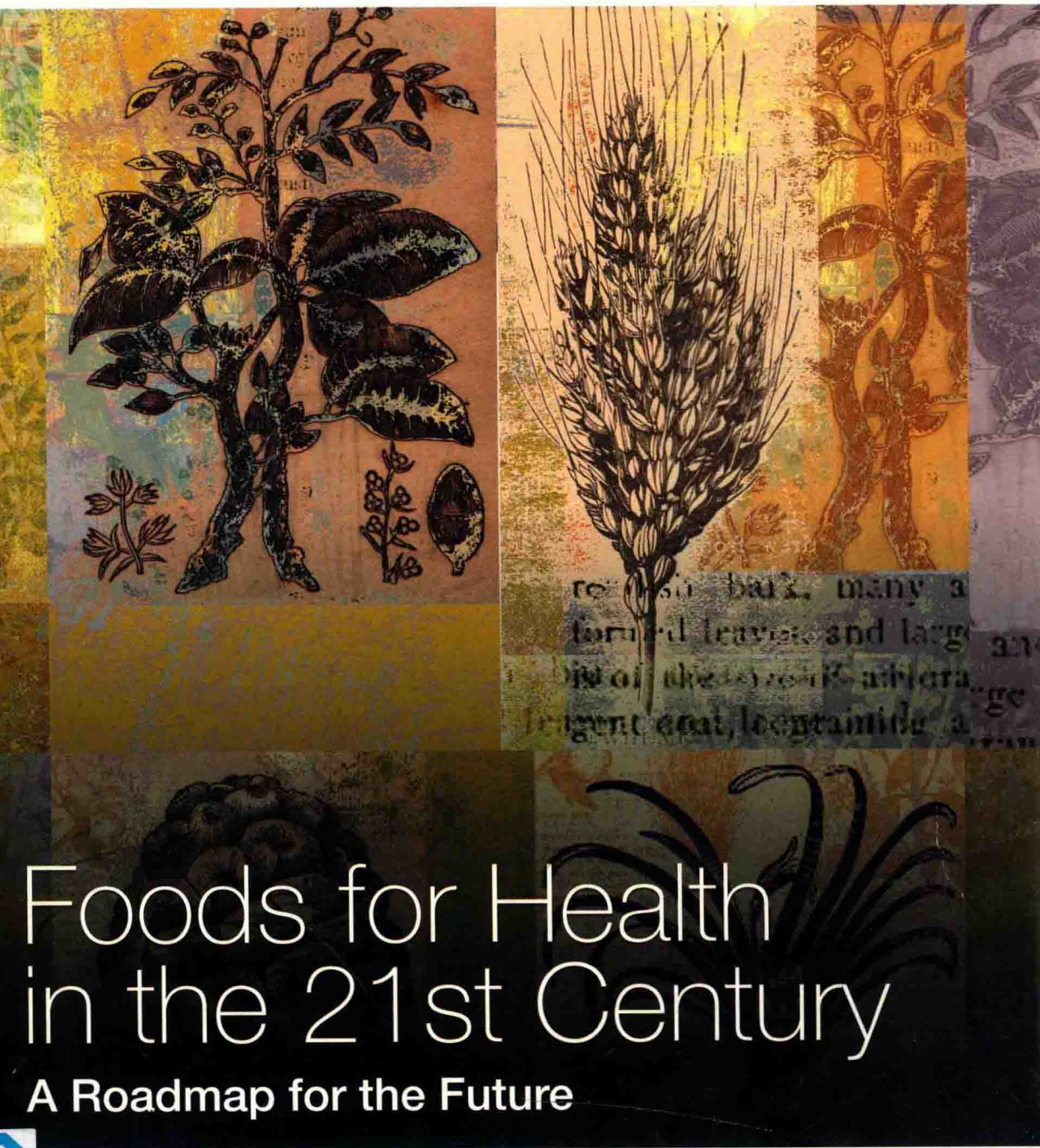

Foods for Health in the 21st Century

A Roadmap for the Future

ISSUE EDITORS

M. Eric **GERSHWIN**

M.R.C. **GREENWOOD**

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Foods for Health in the 21st Century

A Roadmap for the Future

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Introduction

Foods for health: A roadmap for the future

The recognition that dietary constituents play a significant role in not only maintaining good health, but also preventing chronic disease, is a relatively new phenomenon in comparison to the long history of human nutrition and studies of nutritional deprivation. For example, the use of supplemental vitamins, iron, and other minerals has been widespread for more than 50 years. Indeed, Western populations have revolved around the concept that nearly everything can be provided in a pill or a capsule. By contrast, the perception that foods themselves are beneficial and can provide much greater clinical and biochemical improvements to patients than pills is a relatively new finding. Clearly, the whole is better than individual components; that is, consumption of diets rich in antioxidants, such as green vegetables, is far better than taking a dietary antioxidant tablet.

Although this concept may seem obvious to most scientists and physicians, it is still not as widely accepted in the general population as one would expect. Indeed, one has only to go through the shelves in pharmacies and grocery stores to appreciate that, at least, in the United States, more money is spent on dietary supplements than on conventional pharmaceuticals. Indeed, even in those individuals that recognize the importance of foods, there are still misconceptions about how foods should be prepared, the interactions among food ingredients during preparation, and, in particular, the relative absence of the long-term epidemiologic consequences of either a food faddist diet or a diet deficient in specific antioxidants or other food-based biologic therapies. We seem to accept that a vegetarian diet is healthy without any scientific basis, the mechanism of action. Hardly a week goes by when we are not told of the benefits, for example, of green tea, red wine, grapes, or fish, but evidence-based research remains lacking.

On November 16–18, 2008, a conference entitled "Foods for Health in the 21st Century: A Roadmap for the Future" was held on the campus of the University of California, Davis. The goal was to develop a conference that focused on future directions in nutrition and human health, including point–counterpoint presentations that addressed the problems of aging, cancer development, inflammation, immune responses, and the increasing burden of allergies. The conference was hosted by UC Davis but importantly was a collaborative effort with scientists at institutions throughout Denmark. Presentations included work by biochemists, food scientists, engineers, physicians, veterinarians, and particularly physicians involved in new technologies, such as biophotonics, and laboratories that provide novel readouts useful in epidemiologic analysis.

There were multiple specific human health issues defined, including the epidemic rise in obesity, the role of inflammation in chronic fatty liver disease, the role of dairy products, the use of antioxidants in human health, and, especially, major issues in opportunities to improve human health with nutrition. Counterpoint discussions focused on fantasies versus realities and increased international research opportunities.

This *Annals* issue is a collection of papers from a diverse group of scientists. We hope that this volume will be of interest not only to scientists, but also to the general public; it is our sincere hope that this *Annals* issue will reflect the beginning of new efforts to rigorously define the role

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of good nutrition and advance the prevention of human chronic disease. There are many people that helped to produce this volume. In particular, we express our appreciation to Melanie Fumes, associate director of the UC Davis Foods for Health Institute; Nikki Phipps, our editorial assistant; and our many colleagues from Denmark.

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This volume presents papers stemming from the First International Foods for Health Initiative Conference entitled "Foods for Health in the 21st Century: A Roadmap for the Future," held on November 16–18, 2008 at the University of California, Davis Buehler Alumni Center.

Part IV. Functional foods

Part V. Consumer response

Part VI. Emerging technologies

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ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
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The importance of dietary modulation of cAMP and insulin signaling in adipose tissue and the development of obesity

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Adipose tissue plays a pivotal role in whole body energy homeostasis. In this review, we summarize knowledge of the seemingly paradoxical roles of insulin and cyclic adenosine monophosphate (cAMP) signaling in adipocyte differentiation and function, emphasizing the interplay between the two branches of cAMP signaling, the canonical protein kinase A-dependent pathways and the novel exchange protein activated by cAMP (Epac)-dependent pathways, and insulin signaling. We discuss how macronutrients via changes in the balance between insulin- and cAMP-dependent signaling can affect the development of obesity by changing energy expenditure and/or feed efficiency. We review results demonstrating how the balance between different classes of carbohydrates and proteins modulates the obesigenic action of saturated as well as unsaturated fatty acids pointing to insulin as a key determinant in the regulation of the metabolic/regulatory action of both n-3 and n-6 polyunsaturated fatty acids.

Keywords: adipocyte; adipose tissue; obesity; insulin; cAMP

Adipose tissue plays an important role functioning as an energy-depositing organ. Energy is stored in large lipid droplets that represent 95% of the adipocyte volume. Increasing or decreasing the adipose tissue mass is obviously related to energy-intake and expenditure. The latter does, however, not only relate to the level of physical activity, since energy-efficiency may be influenced by a variety of factors, such as energy uptake from the gut, hormonal status, composition of the diet, and genetics. For instance, high-protein diets reduce feed efficiency in both mice[1] and men.[2] In addition, the background diet can determine the adipogenic potential of the dietary fat.[1] Mice fed a high-fat diet in combination with protein had a markedly lower feed efficiency and developed far less adipose tissue mass than mice pair-fed a high-fat diet in combination with carbohydrates.[1] The high-protein-fed mice needed almost seven times more calories to achieve a weight gain of 1 g than mice on the high-carbohydrate diet where the weight gain almost exclusively represented an increase in adipose tissue mass. The high-fat diet in combination with protein resulted into a high glucagon/insulin ratio leading to increased cyclic adenosine monophosphate (cAMP) signaling in adipose tissue.[1] The insulin and cAMP signaling pathways are pivotal in regulation of adipose tissue development and function, and here we discuss their potential as targets for regulation by dietary macronutrients. The nutritional status will also regulate transcriptional activity, and adjustment of gene expression is an important mechanism by which mammals adapt to their nutritional environment. Macronutrients impinge on a number of key regulatory transcription factors involved in adipogenesis and adipocyte function. However, the direct effect of macronutrients on transcriptional regulation in adipose tissue was recently reviewed[3] and will therefore only shortly be dealt with here.

Cyclic AMP and the physiological regulation of adipose development and function

cAMP was the original "second messenger" to be discovered. cAMP-mediated signaling pathways

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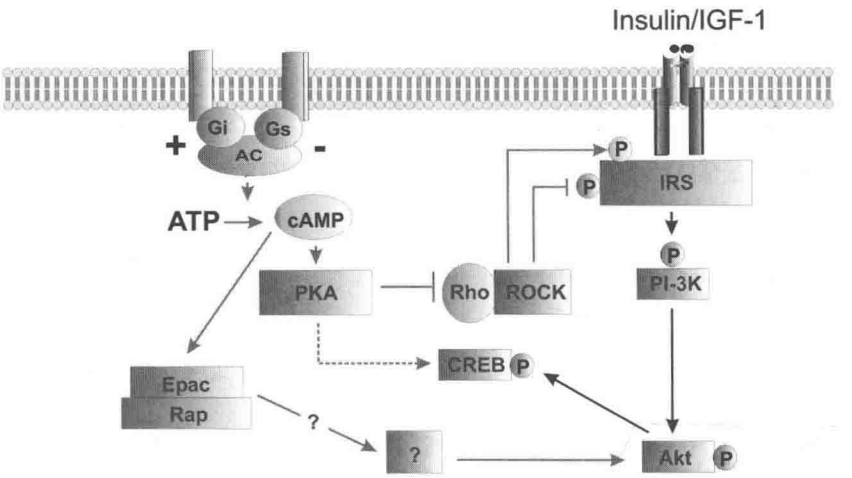

Figure 1. The role of cyclic adenosine monophosphate (cAMP) in insulin/IGF-1 stimulated adipocyte differentiation. Increased levels of cAMP activate both protein kinase A (PKA)- and Epac-dependent pathways. Activation of PKA leads to repression of Rho-kinase activity by targeting either the Rho-kinase or the upstream regulator Rho. High levels of Rho-kinase activity inhibit Ins/IGF-1-dependent signaling, and attenuation of Rho-kinase activity is crucial for adipogenesis. However, low levels of Rho-kinase activity also enhance insulin/IGF-1-dependent signaling, and strong PKA-mediated inhibition of the Rho-kinase thus impairs insulin/IGF-1-dependent signaling. This is counteracted by the simultaneous activation of an Epac1/Rap1-dependent pathway. Activation of cAMP responsive element-binding protein is not dependent on PKA activity, but rather requires ERK activity during the initial stages of adipogenesis. Green and red arrows and Ⓟ indicate signaling and phosphorylation mediated by cAMP and insulin/IGF-1, respectively.

intercede the intracellular actions of several hormones, such as glucagon and epinephrine, and regulate a multitude of important biological processes under both physiological and pathological conditions. Moreover, cAMP functions as a state of starvation signal and mediates hormonal signals from the pancreas and adrenal gland to stimulate glucose production in the liver and lipolysis in adipose tissue.

cAMP and adipocyte differentiation

Increases in adipocyte number occur via proliferation and differentiation of preadipocytes, processes believed to occur throughout life. Research using cell lines, such as 3T3-L1 or mouse embryo fibroblasts, has provided a large amount of the available information on terminal adipocyte differentiation. Treatment of these cells with fetal bovine serum, glucocorticoids, and high levels of insulin or physiological concentrations of insulin-like growth factor-1 (IGF-1) initiates differentiation. However, factors that increase cellular cAMP, strongly accelerate the initiation of the differentiation program by suppression of Wnt10b and Spt and induction of C/EBPβ (for review, see Ref. 4) Moreover, we have demonstrated that the transcriptional activity of peroxisome proliferator-activated receptor (PPAR)δ is regulated synergistically by ligands and cAMP[5] and that cAMP is involved in the production of endogenous PPARγ ligand(s).[6]

The cAMP-responsive element-binding protein (CREB) is another central transcriptional activator of the adipocyte differentiation. Activated CREB induces expression of C/EBPβ, triggering expression of a number of transcription factors, including C/EBPα and PPARγ, of which the latter is the indispensable player in the differentiation program. The importance of CREB is underscored by the finding that adipocyte differentiation of CREB-deficient mouse embryo fibroblast is impaired[7] and that siRNA-mediated depletion of CREB and the closely related activating transcription factor 1 (ATF1) blocks adipocyte differentiation.[8] CREB was initially characterized as a cAMP target whose transcriptional activity was stimulated by cAMP-dependent protein kinase A (PKA)-catalyzed

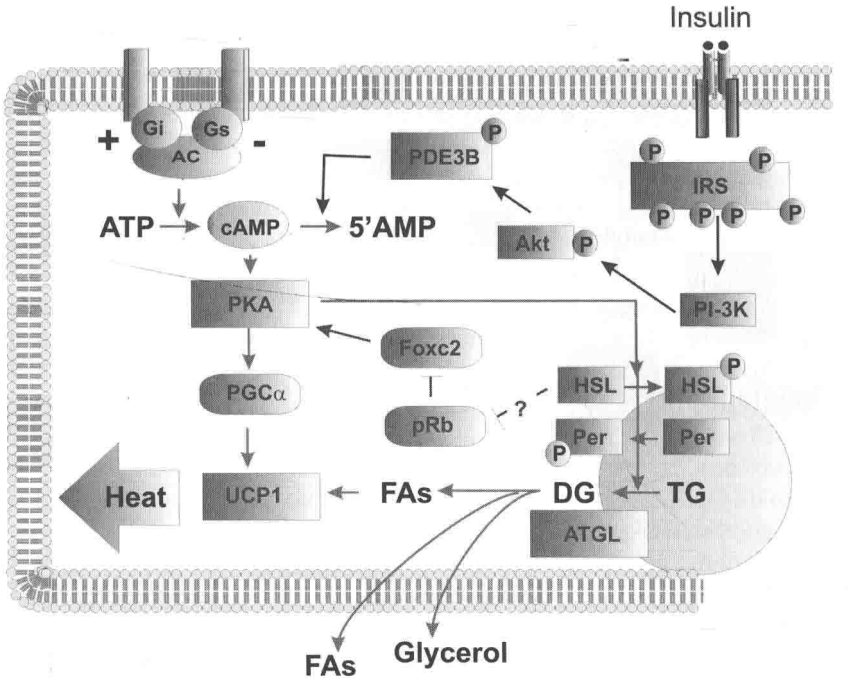

Figure 2. The role of cAMP and insulin in regulation of energy expenditure in brown and brown-like adipocytes. PKA is activated by increased the levels of cAMP as a result of β-adrenoceptor-mediated increase in adenylate cyclase (AC) activity. Increased PKA-activity induces expression of uncoupling protein 1 (UCP1) that uncouples oxidative phosphorylation by dissipating the proton gradient across the inner mitochondrial membrane. Furthermore, PKA stimulates lipolysis by phosphorylating hormone sensitive lipase (HSL) and perilipin (per) and possibly adipose triglyceride lipase (ATGL). Liberated fatty acids (FAs) may thus be β-oxidized. Lack of HSL leads to reduced pRb expression that may stimulate UCP1 expression and sensitize PKA-activity via Foxc2-dependent induction of the regulatory RIα subunit. Insulin disrupts PKA activation by phosphorylation and activation of cyclic nucleotide phosphodiesterase 3B (PDE3B) and modulation of AKAP scaffolding proteins. Insulin signaling is mediated by insulin receptor substrates (IRS), PI3-kinase (PI-3K) and Akt. Green and red arrows and Ⓟ indicate signaling and phosphorylation mediated by cAMP and insulin, respectively.

phosphorylation on serine 133,[9] but insulin signaling may also activate CREB in 3T3-L1 cells through Ser133 phosphorylation via the extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathway.[10] In fact, recent results from our laboratory strongly indicate that PKA activity is dispensable for the increased phosphorylation of CREB during the initiation of adipocyte differentiation.[11] However, PKA activity appears to play an important role for the suppression of Rho/Rho-kinase activity (see below).

While cAMP signaling via PKA has been investigated for decades, the complexity of cAMP signaling via the interplay between PKA and the exchange proteins directly activated by cAMP (Epac1 and Epac2) is only beginning to be understood. Epac1 and Epac2 function as guanine nucleotide exchange factors for the Ras-like small GTPases Rap1 and Rap2 and possibly Rit. We have recently demonstrated that cAMP stimulates adipocyte differentiation *in vitro* through the concerted action of both PKA and Epac/Rap.[11] Activation of PKA inhibits RhoA and Rho-kinase signaling and inhibition of Rho-kinase is essential for adipocyte differentiation.[12] However, strong inhibition of RhoA and Rho-kinase decreases insulin/IGF-1 signaling that is also essential for adipocyte differentiation (see below). We have demonstrated that, concomitant activation of Epac restores insulin/IGF-1 sensitivity when Rho-kinase is inhibited.[11] Thus, in conditions with elevated levels of cAMP resulting in PKA-dependent inhibition of Rho activity,

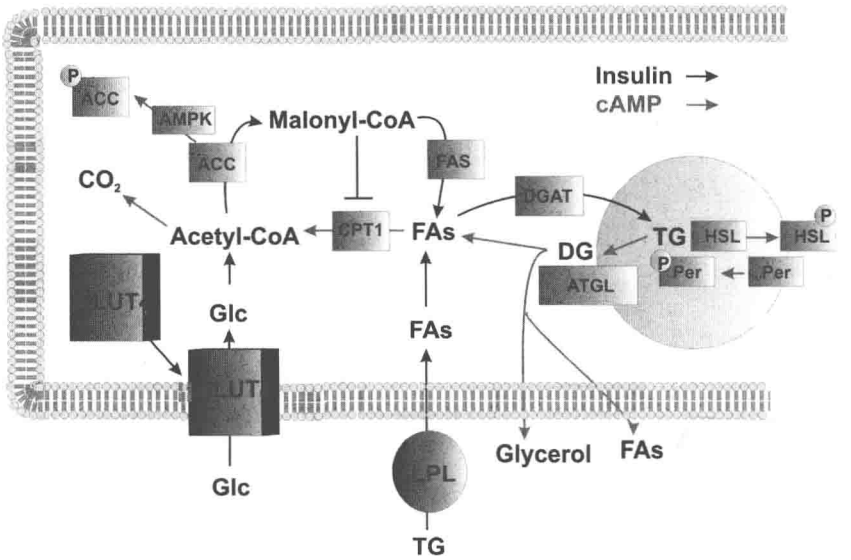

Figure 3. Opposing roles of cAMP and insulin in fatty acid catabolism and anabolism. Increased cAMP levels leads to increased lipolysis by PKA-mediated phosphorylation of HSL and perilipin (per) and possibly ATGL. Liberated fatty acids (FAs) may be released or β-oxidized to acetyl-CoA and further to CO_2. Phosphorylation of acetyl-CoA carboxylase (ACC) by adenosine 5′-monophosphate-activated protein kinase (AMPK) inhibits ACC activity preventing the conversion of acetyl-CoA into malonyl-CoA, an inhibitor of the rate-limiting enzyme in mitochondrial β-oxidation, carnitine palmitoyltransferase (CPT)-1. Insulin stimulates uptake of fatty acids (FAs) by induction of lipoprotein lipase (LPL) and of glucose by enhancing expression and translocation of the GLUT4- transporter. Glucose may be converted to FAs by *de novo* fatty acid synthesis via malonyl-CoA that inhibits CPT1 and mitochondrial β-oxidation allowing efficient accumulation of FA for triacylglycerol (TG) synthesis. Both *de novo* synthesized FAs and FAs taken up from circulation are incorporated into TG, by acyl-coenzyme A: diacylglycerol acyltransferase (DGAT). The transcription factors peroxisome proliferator-activated receptor γ (PPARγ), CCAAT/element-binding protein α (C/EBPα) and sterol regulatory element-binding protein 1 (SREBP1) regulate expression of lipogenic genes. Green and red arrows and Ⓟ indicate signaling and phosphorylation mediated by cAMP and insulin, respectively.

effective insulin/IGF-1 signaling seems to depend on a compensatory sensitizing effect by the Epac branch of the cAMP effector machinery (Fig. 3).

The role of Epac in adipose tissue development and function *in vivo* remains to be elucidated, but given the fact that cAMP is an important second messenger in the regulation of metabolism in adipose tissue, it seems likely that Epac also contributes in cAMP signaling. The RIIβ isoform of PKA is abundantly expressed in adipose tissue with limited expression elsewhere and mice with targeted disruption of the RIIβ isoform of PKA are lean and protected against diet-induced obesity.[13] In this respect it is important to note that loss of the RIIβ protein is associated with a compensatory increase of the RIα isoform, rendering a PKA holoenzyme more easily activated by cAMP.[13] Thus, cAMP signaling through PKA is increased in the RIIβ mutant mice and basal lipolysis is higher in RIIβ-mutant mice than wild-type mice.[13]

cAMP and lipolysis

An important role of cAMP in adipose tissue function is its stimulating effect on the hormone-sensitive lipase and lipolysis. Important stimulators of lipolysis include catecholamines and synthetic β-adrenergic receptor agonists. When agonists bind to β-adrenergic receptors, coupling to adenylate cyclase via stimulatory G-proteins leads to increased cAMP levels. Phosphorylation and activation of cyclic nucleotide phosphodiesterase 3B (PDE3B) on the other hand reduces intracellular cAMP levels. Thus, chemical inhibition of PDE3B increases PKA-activity and lipolysis and PDE3B

knockout mice have reduced adipose tissue mass and reduced adipocyte size.[14]

During fasting, catecholamines activate β−adrenergic receptors leading to increased cAMP levels and activated PKA. PKA stimulates lipolysis by phosphorylating hormone-sensitive lipase (HSL) and perilipin. Whereas HSL directly catalyzes the hydrolysis of triacylglycerol and diacylglycerols, perilipins are proteins that cover the lipid droplets of the adipocytes and protects them from lipolysis. The importance of perilipin is demonstrated by the finding that isolated adipocytes of perilipin null mice exhibit elevated basal lipolysis and the mice are resistant to diet-induced obesity.[15] Isoproterenol-induced lipolysis is blunted in adipocytes from HSL-knockout mice, but basal lipolysis is unaffected.[16] HSL-deficient mice are not obese, and accumulate diacylglycerol in adipose tissue and muscle.[16] This suggests that HSL may be rate-limiting for diacylglycerol hydrolysis and the presence of other lipases, such as adipose triglyceride lipase may compensate for the lack of HSL in adipose tissue. In this respect it should be mentioned that HSL null mice are resistant to diet-induced obesity.[17] However, the lean phenotype of these mice is likely to be related to reduced expression of pRb and RIP140 and increased expression of uncoupling protein-1 (UCP1), in white adipose tissue.[18]

cAMP and white to brown adipocyte transdifferentiation

The lean phenotype of the RIIβ knockout mice, might be in part explained by increased expression of the uncoupling protein UCP1 in both brown and white adipose tissue.[13] UCP1 is an integral mitochondrial inner membrane protein that is induced in a cAMP-dependent manner upon β-adrenergic stimulation and is a hallmark of brown adipocyte mitochondria. It acts as a proton channel, which uncouples oxidative phosphorylation by dissipating the proton gradient across the inner mitochondrial membrane. Thus, energy is lost as heat and an increased abundance of brown adipocytes expressing UCP1 can counteract diet-induced obesity.

Treatment of mice with the β_3-adrenoceptor agonist CL 316243 or cold-exposure elicits strong β-adrenergic stimuli and treatment with β-adrenergic agonists reduces adipose tissue mass. (See review. Ref. 19) In response to cold exposure and treatment with β-adrenergic agonists, mitochondria-rich UCP1 expressing multilocular adipocytes are recruited in white adipose tissue. The origin of these brown adipocytes has been a matter of dispute, but available data now suggest that most of the newly formed brown adipocytes recruited upon β-adrenoceptor agonist stimulation derive from a direct transformation of mature white adipocytes. The importance of β-adrenergic signaling in this respect is documented by the finding that the recruitment of novel brown adipocytes in white adipose tissue after 10 days of cold acclimatization is blunted in β_3-adrenoceptor knockout mice.[20]

Interestingly, as seen in the RIIb knockout mice,[24] both pRb deficiency[42,43] and Foxc2 overexpression[44] result in an increased RIa/RIIb ratio rendering PKA more sensitive to cAMP, leading to an increase in the occurrence of brown adipocytes in white adipose tissue and protection against diet-induced obesity. The induction of UCP1 expression in adipocytes in white adipose tissue has indeed been suggested to play a pivotal role in the protection against obesity. Also, mouse strains that have more UCP1-expressing adipocytes in their white adipose tissue depots are protected against diet-induced obesity (Fig. 2).

Insulin and the physiological regulation of adipose tissue development and function

Insulin is a powerful anabolic hormone that stimulates adipocyte differentiation and adipose tissue expansion. Additionally, insulin exerts a vital control of adipocyte function by inhibiting lipolysis, stimulating *de novo* fatty acid synthesis and uptake of free fatty acids and glucose. Skeletal muscle is the major tissue for insulin-stimulated uptake of glucose, but the uptake of glucose in adipose tissue is significant; a fact underscored by the finding that adipose-specific GLUT4 knockout mice develop glucose intolerance[23] and adipose-specific overexpression of GLUT4 reverses diabetes in muscle-specific GLUT4 knockout mice.[24] Obesity is often accompanied by whole-body insulin-resistance that may include insulin resistance in liver, muscle, and adipose tissue. However, whereas insulin resistance in muscle and liver will cause serious metabolic harm, insulin resistance in adipose tissue may be advantageous as mice lacking insulin receptor in adipose tissue (FIRKO mice) have reduced adipose tissue mass

and extended lifespan/longevity.[25] In this respect, it is worth noting that the FIRKO mice has normal whole-body glucose metabolism, but adipocytes from these mice have normal basal-, but 90% reduced insulin-stimulated glucose uptake.[26] The finding that these mice are protected against age- and hypothalamic lesion-induced obesity strongly suggests that insulin signaling in adipocytes is crucial for obesity development.[26]

Insulin and adipocyte differentiation

The biological actions of insulin in adipose tissue are mediated by tyrosine-kinase activity on the membrane insulin receptors that recruit/phosphorylates insulin receptor substrates (IRS) and Shc docking proteins. Via these initial tyrosine phoshorylations, a complex network transmits insulin signals to initiate downstream signaling. IRS proteins bind to and activate multiple SH2 containing proteins that recognize specific IRS phosphorylated tyrosine residues like the PI-3-kinase leading to activation of Akt, whereas the Shc family of proteins primarily is involved in activation of ERK1/2 kinases and coupled to proliferation. IRS-1 and -2 are the most abundantly expressed members of the IRS family in adipocytes. Signaling trough both IRS-1 and -2 appears to play a role in adipocyte differentiation as the differentiation of IRS-$1^{-/-}$ and IRS-$2^{-/-}$ cells into mature adipocytes is 60 and 15%, respectively, relative to wild-type cells.[27] Mice lacking IRS-1 are growth retarded and insulin-resistant,[28] whereas IRS-2 knockout mice, on the other hand, are obese.[29] The latter might, however, be related to reduced expression of pro-opiomelanocotin in the accurate nucleus accompanied by increased food intake.[29] Actually, also neuron-specific disruption of the insulin receptor gene causes increased food intake and obesity.[30] It should also be mentioned that the IRS-2 knockout mice have decreased expression of the UCP1 in brown adipose tissue,[29] and thus, decreased energy expenditure might account for the susceptibility to obesity development. Cells lacking both IRS-1 and IRS-2 cannot undergo adipocyte differentiation and white adipose tissue mass is reported to be dramatically reduced, but not absent in newborn IRS-$^{-/-}$ IRS-$2^{-/-}$ double-knockout mice.[27]

Insulin-stimulated activation of PI3K and Akt activates a myriad of intracellular responses. PI3K[31] and Akt1,[32] but not Akt2[33] are required for adipocyte differentiation *in vitro*. It should be noted, however, that IGF1-receptors outnumber insulin-receptors 3 to 1 in the preadipocytes. At high concentrations, insulin may bind to both insulin- and IGF1-receptors, but both insulin- and IGF1-stimulated Akt-phosphorylation is impaired in insulin receptor knockout cells.[34] Akt1 is also required for *ex vivo* differentiation of mouse embryo fibroblasts.[33] Akt1/2 double knockout mice die shortly after birth, but it has been demonstrated that newborns lack differentiated adipose tissue.[35] It remains to be elucidated in molecular details why Akt is essential for adipocyte differentiation. However, involvement of mTOR seems plausible. Inhibition of mTOR by rapamycin is known to inhibit differentiation of adipocytes. In particular, a substrate of mTOR, the eukaryotic initiation factor 4E-binding protein-1 (4E-BP1) is of great interest as this factor appears to be a novel regulator of adipogenesis and metabolism.[36] It has been demonstrated that 4E-BP1 is highly induced during differentiation of adipocytes and phosphorylated in response to insulin, and mice lacking this translational inhibitor have less white adipose tissue.[36] On the other hand, mice that lack both 4E-BP1 and 4E-BP2 were recently demonstrated to have increased sensitivity to diet-induced obesity.[37] Given the opposing roles of elevated insulin- and cAMP signaling on metabolism in general, it appears as a paradox that adipocyte differentiation, which indeed requires insulin signaling, is accelerated by cAMP. However, we have demonstrated that activation of Epac enhances insulin/IGF-1-dependent activation of Akt in preadipocytes.[11] Activation of Epac1 potentiated insulin-dependent activation of Akt as well as p70(S6K) in skeletal muscle.[38] Thus, effective insulin/IGF-1 signaling might be dependent on a compensatory sensitizing effect by the Epac branch of the cAMP effector machinery.

Insulin and glucose uptake

Insulin stimulates glucose uptake by inducing GLUT4 translocation and both basal and insulin-stimulated glucose uptake are impaired in adipocytes lacking GLUT4.[23] Vice versa, transgenic expression of GLUT4 in adipose tissue increased basal glucose transport in adipocytes.[39] Adipose tissue-specific GLUT4 knockout mice develop glucose intolerance, but they have normal adipose tissue mass and normal adipocyte size,[23] whereas

transgenic expression of GLUT4 in adipose tissue increase adiposity due to adipocyte hyperplasia.[39]

Interestingly, insulin-stimulated GLUT4 translocation, glucose uptake, and lipogenesis in adipocytes can be inhibited by chemical inhibition of PDE3B.[40] Although inhibition of PDE3B increases PKA-activity, the effect was not abolished by H89. However, the inhibitory effect on GLUT4 translocation was mimicked by activation of Epac.[40] The authors suggested an intriguing possibility that PDE3B controls a cAMP-pool, which through Epac-mediated signaling inhibits GLUT4 translocation and insulin-stimulated glucose uptake.[40]

Insulin and lipolysis

Insulin is probably the most important antilipolytic hormone. Insulin is able to depress hormone-stimulated adenylate cyclase and disrupt β-adrenergic signaling to PKA in adipocytes by weakening the apposition of β-adrenergic receptors and PKA through modulation of AKAP scaffolding proteins.[41] Moreover, insulin is able to lower intercellular cAMP-levels by activation of PDE3B.[42] The role of insulin receptors is supported by the fact that insulin fails to suppress lipolysis in adipocytes lacking the insulin receptor.[26] Insulin also increases expression of the adipocyte phospholipase A2 (AdPLA), the major PLA2 in adipose tissue.[43] This is of interest as ablation of AdPLA leads to increased lipolysis and protection against obesity induced by both diet and leptin deficiency.[43]

Insulin and gene expression

Insulin is an important regulator of the sterol regulatory element-binding proteins (SREBPs). The SREBPs constitute a family of transcription factors involved in regulation of intracellular lipid homeostasis. The expression of lipogenic genes is controlled by the transcription factors SREBP-1a and -1c, but the nutritional induction of genes involved in lipogenesis is mainly controlled by the SREBP-1c isoform. The majority of the work involving SREBP and lipogenesis concerns the liver, but SREBP is known to regulate the expression lipogenic genes also in adipocyte cell lines and adipose tissue. (see review. Ref. 44) SREPB-1c is the predominant form in adipose tissue. However, whereas adipocyte-specific overexpression of SREBP-1c in mice strongly inhibits adipocyte differentiation and renders the mice lipodystrophic,[45] adipocyte-specific transgenic expression of SREBP-1a in mice leads to massively enlarged adipocytes with increased expression of lipogenic genes and increased rate of *de novo* fatty acid synthesis.[46] Interestingly, refeeding fasted animals a fat-free, high-carbohydrate diet induces *de novo* fatty acid synthesis and expression of SREBP-1c and lipogenic genes to levels significantly higher than those observed in the normal fed state in adipose tissue.

SREBP-1c plays an essential role in mediating the liver X receptor (LXR)-response as the increased expression of lipogenic genes in mice treated with LXR agonists is blunted in SREBP-1c knockout mice.[47] Also, LXRα/β-deficient mice have reduced expression of SREBP-1c and lipogenic genes.[48] Lipogenesis in adipocytes requires glucose that is supplied by transport through the insulin-responsive glucose transporter GLUT4 that is also directly regulated by the LXRs. A Sp1 element and a functional sterol-response element are also found in the GLUT4 promoter, and thus insulin-stimulated GLUT4 expression may be directly activated by both SREBP-1c and LXR in adipocytes.

The role of *de novo* fatty acids synthesis in adipose tissue in obesity is not clear, but has been suggested to make a quantitatively substantial contribution to the accrual of adipose tissue mass in rodents on low-fat diets. Moreover, *in vivo* ^3H_2O labeling studies have demonstrated increased *de novo* lipogenesis in the obese *ob/ob* mice compared to their lean littermates.[49] It should be mentioned, however, that there seems to be a dissociation between adipose tissue flux through lipid anabolic pathways and gene expression as increased *de novo* lipogenesis is not always followed by high expression of lipogenic enzymes.[49]

Stearoyl-CoA desaturase (SCD) that catalyzes the critical commitment step in the biosynthesis of monounsaturated fatty acids from saturated fatty acids appears to have a unique role in triacylglycerol formation and body weight regulation.[50] Mice with a targeted disruption in the SCD1 gene have increased energy expenditure, reduced body adiposity, increased insulin sensitivity and are resistant to diet-induced obesity.[51] Lack of the SCD1 gene amplifies the signal leading from β3-adrenergic receptor activation to phosphorylation of CREB and induction of PGC1α, which mediate the induction of UCP1 expression in BAT resulting in dissipation of energy as heat.[51] The role of SCD1 specifically in adipose