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Enzymatic intracrine regulation of white adipose tissue

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Abstract

Abdominal fat formation has become a permanent risk factor for metabolic syndrome and various cancers in one-third of the world's population of obese and even lean patients. Formation of abdominal fat involves additional mechanisms beyond an imbalance in energy intake and expenditure, which explains systemic obesity. In this review, we briefly summarized autonomous regulatory circuits that locally produce hormones from inactive precursors or nutrients for intra-/auto-/paracrine signaling in white adipose depots. Enzymatic pathways activating steroid and thyroid hormones in adipose depots were compared with enzymatic production of retinoic acid from vitamin A. We discussed the role of intracrine circuits in fat-depot functions and strategies to reduce abdominal adiposity through thermogenic adipocytes with interrupted generation of retinoic acid.

Keywords

aldehyde dehydrogenase; endocrine; insulin resistance; nuclear receptors; Raldh1

Introduction

The number of cases of obesity has nearly doubled over the past two decades, according to the World Health Organization. Obesity can be defined by a body mass index (BMI) of 30 or greater, which is calculated by the individual's weight in kg divided by height in square meters. In the US, approximately 35.7% of adults and 17% of children aged 2–19 are classified as obese (CDC.gov). In patients, obesity often develops in conjunction with the five other metabolic risk factors – abdominal obesity, atherogenic dyslipidemia, elevated blood pressure, insulin resistance (glucose intolerance), prothrombotic and proinflammatory states – that are defined as metabolic syndrome by the American Heart Association [1]. The development of obesity is commonly viewed as a process associated with a chronic energy imbalance where the dietary energy intake is greater than energy expenditure [2]. We will refer to this concept as the ‘overall energy balance’ hypothesis in this review. Physical activity remains the most recommended remedy for obesity because muscles expend energy and can improve energy balance. However, the ‘overall energy balance’ hypothesis only

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partially explains the obesity phenomena. Given systemic effects of diet and physical exercise, it is still inexplicable why fat accumulates differently amongst fat depots and acquires different properties. The understanding of the mechanisms responsible for fat depot formation appears to be a critical issue, because abdominal obesity greatly increases risks for metabolic syndrome, type 2 diabetes, coronary heart disease, stroke, and certain cancers [3, 4]. The propensity to develop abdominal obesity and other metabolic risk factors appears to depend on heritable genetic factors [5, 6], endocrine status [7, 8], and specific ‘zero calorie’ micronutrients [9]. The abdominal and other fat depots have autonomous regulatory circuits that locally produce hormones from inactive precursors or nutrients for intra-/auto-/paracrine signaling (referred to as ‘intracrine’ circuits in this review). In this review, we will discuss recent advances in understanding the role of intracrine circuits in fat depot functions (Figure 1).

Adipose tissue depots

Adipose tissues comprise two predominant types: white adipose tissue (WAT), and brown adipose tissue (BAT) [6, 10]. Although both types of adipose tissue influence obesity, they have opposite functions in energy balance regulation. In BAT, energy from nutrients (glucose and fatty acids) can be partially converted into heat in a process known as thermogenesis [11]. This process is driven by mitochondrial uncoupling protein 1 (UCP1). BAT has numerous UCP1 positive mitochondria that dissipate energy. BAT and skeletal muscle arise from a similar progenitor expressing myogenic factor 5 (*Myf5*) [12]. Neonates have notable amounts of BAT to support thermogenesis [13]. BAT mass declines with age. In adults, only small BAT depots are located in the neck and peri-vascular areas [14]. An elevated amount of BAT is associated with resistance to obesity and improved glucose sensitivity in human and mouse obesity models [11, 15].

WAT is lipogenic and stores energy in the form of triglycerides [16]. There are different types and locations of WAT depots, each having its unique heritable gene expression pattern [5, 17]. Each fat depot exerts systemic effects on energy homeostasis and inflammation [10]. WAT depots are named based on their location. Subcutaneous (also known as peripheral or inguinal) fat depot is found beneath the skin. Abdominal fat accumulates around organs (viscera) and is broadly described as visceral fat. In humans, abdominal fat is subdivided into omental and/or mesenteric fat depending on its developmental origin [5]. Abdominal fat depots are considered to have the most deleterious health consequences because of the production of proinflammatory cytokines [18] and hormones (e.g., cortisol) in this tissue [11]. However, little is known about genetic and intracrine mechanisms that can diversify inflammatory responses among the different fat depots.

WAT can also contain thermogenic adipocytes expressing *Ucp1* and *Ucp2* [19]. We describe these adipocytes as ‘thermocyttes’ in this review, while they have also been termed as ‘brown-like’, ‘beige’ or ‘brite’ adipocytes elsewhere [19]. White thermocyttes and lipogenic adipocytes in WAT appear to originate from a similar precursor; however, they express distinct gene clusters [20]. In WAT, thermogenesis can be induced by either sympathetic nerve stimulation, prolonged cold exposure [21], or consumption of high-fat and spicy diets [22]. Induction of thermogenesis in WAT can change energy homeostasis and effectively

decrease obesity and insulin resistance [9, 23, 24]. Notably, the increased proportion of thermogenic to lipogenic adipocytes in abdominal fat can decrease lipid accumulation in this deleterious depot [23]. Although thermogenesis in WAT offers a unique therapeutic opportunity to decrease abdominal fat, the current methods of inducing thermogenesis in WAT are not suitable for clinical application. Prolonged stimulation of β -adrenergic receptors mimics the action of epinephrine and norepinephrine, and can impair cardiac function [25]. Extended cold exposure or spicy diets have transient thermogenic effects and eventually increase weight in patients, thereby, having no feasible clinical application. Recent studies demonstrated that enzymes in WAT can effectively convert hormone precursors or micronutrients into intra-/paracrine hormones or catabolize them [26]. These enzymatic processes mediate fat-depot-specific effects of hormones that participate in formation of fat depots, thermogenic remodeling, and inflammation. In our review, we will briefly summarize the role of enzymes that regulate production of hormones from precursors and micronutrients in WAT (Figure 1). Specifically, we describe the function of hormones regulating the nuclear receptor family of transcription factors in WAT. We will speculate how regulation of enzymatic activity can help to achieve fat-depot-specific therapeutic effects.

Cortisol metabolism in WAT

The glucocorticoid hormone, cortisol, is produced by the adrenal cortex. In kidney and other mineralocorticoid target tissues, cortisol is inactivated to cortisone, whereas WAT and other peripheral tissues convert cortisone to cortisol (reviewed in [27]). Cortisol and cortisone are transported through the blood in healthy subjects at a ratio of approximately 3:1 [28]. Cortisol acts principally through two nuclear receptors: glucocorticoid (GR) and mineralocorticoid receptors (MR) (Figure 2) (reviewed in [29]). The evidence of systemic cortisol effects on WAT formation was established in patients treated with synthetic and natural glucocorticoids and from patients with Cushing's syndrome [30]. Cushing's syndrome was initially described as cortisol overproduction in patients with tumors on the adrenal gland, whereas now this term is commonly used to describe conditions associated with high plasma levels of cortisol. In these patients, an excess of cortisol in blood induces abdominal obesity among other metabolic morbidities [30–33]. In healthy and obese patients, blood levels of cortisol did not correlate with obesity [34–38]. Normal cortisol levels are found even in patients with abdominal obesity [34]. Thus, systemic levels of cortisol in plasma cannot be considered as a marker or factor that induces abdominal obesity in otherwise healthy adults [39].

In WAT and other peripheral tissues, cortisol is generated from inactive forms by 11β -hydroxysteroid dehydrogenase type 1 (11β -HSD1); however, under specific conditions this enzyme also catalyzes a reverse reaction [27, 40, 41]. In mice, 11β -HSD1 predominantly catalyzes conversion of 11-dehydro-corticosterone into active corticosterone. Cortisol can be inactivated to cortisone by 11β -hydroxysteroid dehydrogenase type 2 (11β -HSD2) in the WAT [39]. The link between 11β -HSD1/ 11β -HSD2 and obesity was established in Dr. Jeff Flier's laboratory [7, 40, 41]. Transgenic mouse models overexpressing 11β -HSD1 in WAT exhibited abdominal obesity and a spectrum of associated metabolic disorders, such as insulin resistance and high blood pressure [40, 41]. Overexpression of 11β -HSD1 in brown

adipocytes resulted in decreased levels of UCP1 that reduced energy expenditure in this tissue [42]. In the abdominal fat of 11 β -HSD1^{-/-}, preadipocytes have impaired adipogenesis compared to wild-type preadipocytes [43]. It is assumed that 11 β -HSD1 effects depend on cortisol activation of GR [44]; however, WAT express both GR and MR during differentiation in a biphasic fashion [45]. Contribution of these receptors to 11 β -HSD1 effects in WAT remains to be deciphered.

Human studies generate conflicting reports on the role of 11 β -HSD1 in abdominal obesity. In a 2002 study, BMI in female volunteers did not correlate with 11 β -HSD1 expression in omental fat [46]. Furthermore, 11 β -HSD1 expression was similar in visceral and subcutaneous fat samples [46]. Oddly, cortisol decreased proliferation of omental preadipocytes, while 11 β -HSD1 expression was reduced [46]. More recent findings showed that 11 β -HSD1 expression is similar among groups of severely obese women with and without metabolic syndrome and lean women [47]. In contrast, other studies using visceral and subcutaneous biopsies of lean and obese women found that expression levels of 11 β -HSD1 were greater in obese than lean groups [48]. In obese populations, expression levels of 11 β -HSD1 were higher in omental than subcutaneous fat [49–52]. 11 β -HSD1 expression levels and activity in subcutaneous fat are increased after menopause, however, the relevance of this finding in abdominal obesity is unclear [53]. 11 β -HSD1 expression in omental fat explains approximately 20% of the variance in abdominal fat deposition in postmenopausal women [54].

The inconsistencies in data linking hypercortisolism or dysregulation of 11 β -HSD1 to abdominal obesity can evolve from different factors. The preferential abdominal fat depot formation by 11 β -HSD1 was also explained by fat-depot-specific cortisol excretion from WAT [52]. Abdominal adiposity in patients correlates with urinary cortisol excretion rates (reviewed in [39]). In healthy men, cortisol is released from subcutaneous fat depots, whereas no secreted cortisol from abdominal fat depots was detected [52]. The retention of cortisol in abdominal fat can potentially activate cortisol-dependent pathways and increase abdominal obesity. The mechanisms underlying different cortisol excretion pathways and function in different fat depots were not investigated. Studies in bariatric patients showed a decrease of 11 β -HSD1 expression and cortisol secretion after weight loss [55], suggesting that regulation of 11 β -HSD1 pathway may be dependent on intrinsic factors within adipose tissue. 11 β -HSD1 expression is increased by NF- κ B, a key mediator of inflammation, and decreased by hypoxia inducible factor-1 α (HIF-1 α) [56]. Inactivation of cortisol to cortisone by 11 β -HSD2 also plays an important role in diversification of subcutaneous and visceral fat responses. In male Wistar rats fed a high-fat diet, 11 β -HSD2 expression is greater in the subcutaneous fat than in the visceral fat [57]. In this study, 11 β -HSD2 expression was correlated with both adiposity and insulin resistance. This correlation suggests that cortisol inactivation controls cortisol availability for intracellular signaling. Consistent with these observations, mice overexpressing 11 β -HSD2 in adipose tissue resist high-fat diet-induced obesity and insulin resistance [7]. The changes in 11 β -HSD2 levels in these animals have both systemic and local effects on metabolism, via decreased production of cytokines (leptin and resistin) and up-regulation of thermogenic *Ucp2*. However, it has been hypothesized that overexpression of 11 β -HSD2 in other tissues, such as kidney, increases systemic

cortisone levels for utilization by the adipose tissue [39]. In spite of encouraging data in animal models, the targeting of 11 β -HSD1/11 β -HSD2 pathways for treatment of visceral obesity may cause significant side effects in other tissues because adipose tissue substantially contributes to the systemic pool of cortisone/cortisol.

Deiodinase functions in adipose tissue

Thyroid hormones (iodothyronines) play a dominant role in the regulation of energy balance. Two iodothyronines are associated with metabolic regulation: thyroxine (T4) and 3,5,3'-triiodothyronine (T3). They are produced in the thyroid gland (Figure 3). The average daily secretion of thyroid hormones is equivalent to 100 μ g of T4 and 6 μ g of T3 [58]. T4 synthesis is specific to the thyroid gland. T4 functions as a prohormone substrate that generates T3 and its isomer 3,3',5'-triiodothyronine or reverse T3 (rT3) [59]. Approximately 20% of T3 can be secreted directly from the thyroid gland. From circulation, thyroid hormones are translocated across lipid bilayers by several members of the major facilitator superfamily of proteins, including monocarboxylate transporters, amino acid transporters, and organic anion transporting polypeptides [60–62]. Synthesis of T3 predominantly occurs through peripheral monodeiodination of T4 in peripheral tissues including WAT [59]. The rate at which T4 is converted to T3 is dependent on tissue-specific T3 needs [63] and is mediated by enzymes: type I 5'-deiodinase (D1), type II 5'-deiodinase (D2) and type III deiodinase (D3). The deiodinases convert T4 into the products T3 and rT3, as well as T3 and rT3 into 3,3'-T2 (T2), thereby regulating local and systemic availability of iodothyronines (Figure 3). Clinical studies have unsuccessfully attempted to connect hypothyroidism and obesity. Research has shown that <10% of obese individuals have hypothyroidism, with patients maintaining normal T4 (free, total), T3 (free, total) and TSH levels. The exposure to excess energy (overfeeding) resulted in an increase in total T3 and reduction in rT3 [64]. Vice versa, hypocaloric diets in obese individuals provoked suppression of T3, elevation of rT3, and an increase in cortisol levels [64]. Measurements of circulating thyroid hormones seemed to be inconclusive for determining the relationship between thyroid function and increased adiposity.

Thyroid hormones mediate cellular functions through both genomic and non-genomic pathways [59]. T3 is a ligand for the thyroid nuclear receptors (TR α and TR β), which are transcription factors regulating genomic pathways (reviewed in [65, 66]). T3 has four-fold higher affinity to TR than T4. In the nucleus, T3 binds to its cognate TR. Upon nuclear receptor activation, conformational changes occur and result in the formation of homodimers or heterodimers with retinoid X receptor (RXR). TR homo- or heterodimers with RXR interact with thyroid hormone response elements (TREs) in the regulatory regions of target genes. Heterodimer arrangement of TR-RXR is the most transcriptionally active [67]. TR α is expressed in subcutaneous and visceral WAT and other tissues, such as brain, skeletal muscle, kidney, liver and heart. TR α is expressed at higher levels in subcutaneous compared to omental fat depots in women [68]. In this study, expression of TR α was higher in stromal vascular fractions than in mature adipocytes in any fat depots, suggesting a role of TRs in preadipocytes. Although both TR α and TR β are expressed during adipogenesis [45], their role in this process has not been defined. In loss-of-function mouse models with dominant negative mutations of TR α and TR β , researchers have found similar adipose-depot

formations, impaired hepatic lipid metabolism [69], and other systemic effects associated with metabolic rate (reviewed in [70]). Consistent with these animal studies, obesity is associated with a mild increase in subcutaneous TR α expression in lean vs. morbidly obese patients [71]. In this study, weight loss resulted in a 70% increase in TR α expression, possibly as a result of systemic improvement of metabolism in these patients. Association of TR α gene variances with risk of developing obesity is specific to patients' cohorts with or without cardiovascular disease [72]. TR β is expressed in BAT [73], WAT [74] and other tissues such as the kidney, liver, brain, heart, thyroid, skeletal muscle, lungs, and spleen [75]. In WAT, TR β is transiently expressed during early adipogenesis [74]. Its expression can be induced by T3 and PPAR γ agonists [45, 76]. Recent studies demonstrate that in WAT and BAT, TR β mediates adaptive thermogenesis and selective TR β ligands prevent high-fat diet-induced obesity [76, 77]. T3 and 'non-classical' thyroids, such as rT3, T2 and decarboxylated triiodothyroacetic (Triac) and deaminated tetraiodothyroacetic (tetrac) acids, can also act via non-genomic pathways (reviewed in [59]). Triac is a weak agonist for TR β , however, it is the more potent (10–50 times) inducer of thermogenesis, D3 activity, and D3 expression in BAT than T3 [78]. In vivo, picomolar concentrations of bioactive T2 regulate expression of multiple T3-related genes that alter metabolic rate, up-regulation of ATP synthase subunits, and adaptive thermogenesis [79–82]. Through tissue-specific expression of TRs and thyroid-producing deiodinases, tissues can customize their metabolic responses.

Expression of deiodinases is tissue-specific (reviewed in [8]). D1 is ubiquitously expressed in tissues, including WAT, liver, kidney, and thyroid gland [83]. D1 catalyzes removal of iodine from both the inner and outer ring of T4 [63]. D1 activates T4 to T3, or irreversibly inactivates T3 to 3,4-diiodothyronine (T2) [8] (Figure 3). The D1 catalytic activity depends on the rare amino acid selenocysteine [83]. The second isozyme, D2 is expressed in BAT, WAT, skeletal and cardiac muscle, CNS, brain, pituitary gland, and the placenta [8]. D2 plays a predominant role in T3 production via deionization on the outer ring of T4 [83]. The final isozyme D3, controls T3 concentrations by preventing T3 inactivation or by initiating T3 degradation via deionization of the inner ring [63, 83]. Thus, while D1 and D2 are important for conversion of T4 to active T3, D3 instead inhibits this reaction. Inactivation of T3 is the result of the removal of iodine from the inner ring, leading to the production of inactive rT3. Another product of T3 or rT3 deiodination is T2 [79].

Dr. Antonio Bianco and co-workers advanced the fundamental understanding of D2 functions in adipose tissue by developing whole body and tissue-specific D2 mouse models ([84, 85], reviewed in [8]). Consistent with the pivotal role of thyroid signaling in thermogenesis, D2^{-/-} mice housed in thermo-neutral (30°C) environments display traits favoring obesity, glucose intolerance, and hepatic steatosis [84]. However, at room temperatures (22°C), a shift to fat utilization protects D2^{-/-} from high-fat diet-induced obesity [84] probably because of induction of β -adrenergic thermogenic shivering [84]. These findings allude to the key role of D2 in the regulation of basal metabolic rate and adaptive thermogenesis. Paradoxically, D2^{-/-} mice maintained normal T3 and elevated concentrations of T4 and TSH in circulation with markedly reduced cerebral D2 levels. Thus, D2 caused systemic effects via secretion of thyroid hormones in the circulation as well as by direct regulation of hypothalamic-pituitary thyroid axis (reviewed in [86]). Nonetheless, studies in adipose-specific D2^{-/-} mice showed that D2 action in adipose tissue

is sufficient to regulate energy balance and prevent high-fat diet-induced obesity via up-regulation of fatty acid oxidation in BAT at 22°C [85]. In addition, D2 deficiency in adipose tissue influences obesity by controlling systemic responses such as food intake [85]. It is currently unclear whether food intake is influenced through changes in thyroid hormone composition and levels in the circulation. Hypothetically, adipokines produced by adipose tissue in D2^{-/-} mice also can participate in the regulation of these responses. Acute (=4 day) and chronic (7 week) exposure to cold environments (4°C) can induce D2 expression in BAT. This induction increases extrathyroidal T3 by three-fold and T4 levels by 1.4-fold [87]. Thus, it is likely that modulation of deiodinase activities in WAT does not cause tissue-specific effects but rather leads to systemic changes in thyroid-dependent pathways. Reliance on environmental temperature to induce thermogenesis also limits the application of deiodinases for obesity therapies.

Aromatase enzyme complex (Ar) functions in adipose tissue

Estrogens are steroid hormones that regulate major reproductive functions in women. Estrogen functions include gonadotropin secretion for ovulation, preparation of tissues for progesterone response, and control development of secondary sexual characteristics. Estrogens also have metabolic effects that influence both sexes. Estrogens mediate preferential distribution of fat into subcutaneous depots, regulate bone mass, lipoprotein synthesis, and insulin responsiveness to maintain cognitive function [88]. Estrogens are comprised of three major natural forms: estrone (E1), estradiol (E2) and estriol (E3). The principal mechanism of estrogen action is via binding to nuclear receptor estrogen receptors alpha (ER α) and ER β (reviewed in [89]). Among estrogens, E2 has the highest affinity to ER α and ER β followed by E1 and E3 (Figure 4). E2 binding leads to formation of ER α and ER β homodimers on ERE regulatory sequences, a recruitment of co-activators that result in transcription of target genes [90, 91]. E2 also has four-fold higher affinity for binding to ER α than to ER β [90]. The specificity of estrogen action in tissues and fat depots depends on both tissue-specific expression of ER α and ER β and a site of estrogen production. The importance of activation of ER in WAT by locally produced hormones was discovered in context of breast cancer.

The main site of production of E2 and E1 varies depending on age, pregnancy status, and adiposity. In women of reproductive age, the majority of estrogens are produced by glandular organs such as the ovaries and placenta during pregnancy, whereas extraglandular estrogen production in WAT varies in lean and obese women [88, 92]. In postmenopausal women and elderly men, extra-glandular estrogen production in WAT and other peripheral tissues predominates. The biosynthesis of estrogen in WAT is catalyzed by the aromatase enzyme complex (Ar). Ar expression in the adipose tissue is specific to humans, certain primates and rodents [92, 93]. Ar is composed of two polypeptides, the NADPH-cytochrome P450 reductase and the aromatase cytochrome P450 or P450arom (EC 1.14.14.1), which is the product of the *Cyp19A1* human gene. Ar is located in the endoplasmic reticulum, where this enzymatic complex catalyzes the conversion of C19 steroids, such as androstenedione, to C18 steroid estrone (reviewed in [88, 93]) (Figure 4). Ar transcript is different between glandular and extraglandular tissues, and also varies between breast and subcutaneous WAT depots [93]. Sequence specificity of Ar includes regulatory promoter regions that alter Ar

expression in response to endocrine hormones and signaling molecules (reviewed in [94]). This specific regulation determines the tissue- and fat depot pattern of Ar expression.

In WAT, Ar is expressed in stromal vascular fraction and preadipocytes/fibroblasts, whereas mature adipocytes have negligible expression and activity of Ar [93, 95, 96]. However, mature adipocytes from obese insulin-resistant patients produce TNF α and prostaglandin E2 [97, 98] that can potentially induce expression and activity of Ar in WAT depots of obese patients [92]. The estrogens produced by Ar in WAT act locally in an intracrine or paracrine fashion. Both subcutaneous and omental WAT depots also express 17 β -hydroxysteroid dehydrogenase (17 β -HSD) that catalyzes the conversion of E1 into E2 (type 3 17 β -HSD) or the reverse reaction (type 2 17 β -HSD) [99] (Figure 4). Type 3 17 β -HSD is co-expressed with Ar in visceral WAT, where the ratio of 17 β -HSD to Ar expression is positively correlated with BMI and abdominal obesity in women [100]. This ratio in subcutaneous WAT inversely correlates with BMI, indicating depot-specific effects of Ar and local production of estrogens. The activation of ER α in women's subcutaneous fat has been associated with inhibition of the lipoprotein lipase activity and fat intake by increasing the number of antilipolytic α 2A-adrenergic receptors [101]. This mechanism contributes to the preferential formation of subcutaneous fat in premenopausal women. The altered fat distribution in obese women or during pregnancy also coincides with the changes in expression levels of Ar and type 3 17 β -HSD [102].

An insight into the systemic role of Ar in development of WAT has been obtained in aromatase knockout (ArKO) mice [103]. Both male and female ArKO mice show an increase in abdominal fat depots, especially in perigonadal and perirenal regions, when compared to wild-type (WT) mice. The adipocytes of the ArKO mice were found to be of greater volume than in WT mice [104], this effect was attributed to elevated expression of lipoprotein lipase (LPL) in ArKO vs. WT mice at 1 year of age. LPL hydrolyzes triglycerides into free fatty acids and glycerol. The free fatty acids are then taken up by fat cells and esterified into new triglycerides for storage. Treatment of ArKO mice with E2 decreased both LPL activity and adipocyte volume, suggesting LPL regulation by E2. However, Ar influenced WAT in ArKO primarily via systemic regulation of energy balance, such as decrease in spontaneous physical activity and lean body mass in ArKO vs. WT mice. In addition, ArKO mice have impaired glucose oxidation, lipid accumulation in the liver, and other metabolic abnormalities (reviewed in [105]). These effects of Ar were mediated by E2, as administration of E2 to female ArKO mice restored their fat depots to masses comparable to those of WT mice [104]. In humans, loss-of-function mutation in *Cyp19A1* also leads to abdominal obesity and metabolic syndrome [106]. The activity of Ar in preadipocyte cultures is also affected by the cellular hormonal milieu and depends on the type of adipocytes, because adipocytes from different WAT depots are regulated by different promoters. The human *Cyp19A1* gene comprises nine coding exons (reviewed in [107]). Alternative proximal I.3 and II promoters are expressed in breast WAT near to cancerous tumors, whereas I.4 promoter is expressed in all WAT depots [93, 94]. Expression of a single copy of the human Ar in transgenic mice is sufficient to develop breast hyperplasia [108].

Physiological regulators of Ar expression and activity in WAT are glucocorticoids (mainly dexamethasone and cortisol), cAMP analogues, and phorbol esters [93, 96]. Sex-hormone binding globulin (SHBG) could be considered as a critical factor in limiting Ar activity and/or systemic effects of Ar, because it transports the sex-steroid hormones in the blood (reviewed in [109]). In agreement, SHBG concentrations in blood inversely correlate with the abdominal fat mass in premenopausal women [110]. Dietary flavonoids and lignans, such as enterolactone, enterodiol and other phytoestrogens [111], have structural similarities with estrogen and can bind to ERs, preventing the binding of estrogens to the ERs and inhibiting Ar activity [112].

Although Ar in adipose tissue can contribute to systemic effects via cytokine production, it appears that the majority of tissue-specific effects are regulated in a paracrine manner. Ar function in WAT creates a critical microenvironment for breast cancer cells (reviewed in [94]). Expression of *Cyp19A1* in the adipose tissue of breast cancer subjects is higher at regions proximal to the tumor sites as compared to distal regions. The expression and subsequent activity of Ar is also induced by a higher activation of the retinoic acid receptor-related orphan receptor α (ROR α) in breast cancer cells [113] and by retinoic acid via retinoic acid receptor (RAR) α in human adipocytes [114]. ER β is also expressed in the human adipose tissue in men and women and its expression has been associated with breast cancer [115]. Because *CYP19A1* produces estrogen in the breast adipose tissue, it has been proposed that Ar expression and activity leads to breast cancer and endometrial cancer [92, 93]. Estrogen biosynthesis by Ar has been demonstrated to be greater in obese patients as a result of elevated adipose tissue mass. Numerous clinical studies provide unequivocal evidence that obesity increases three-fold the risks for breast cancer in postmenopausal women (reviewed in [94]). Anastrozole – inhibitor of Ar activity – appears to be more efficient in prevention of recurrence of breast cancer with fewer side effects than ER antagonists [116]. These studies have also shown the functional role of Ar in WAT, because this Ar inhibitor tends to increase weight gain in breast cancer patients [117]. It remains unstudied how Ar inhibition influences fat distribution in these patients. Thus, while glandular organs play a dominant role in biosynthesis of estrogen, the local production of estrogen in WAT depots only becomes pivotal for regulation of fat depots in postmenopausal women.

Intra/paracrine effects of retinoic acid production from vitamin A in WAT

The pathway converting vitamin A into retinoic acid (RA) for regulation of WAT is principally different from those activating steroid and thyroid hormones from prohormones.

1. Vitamin A is converted to RA exclusively in extraglandular tissues.
2. **WAT stores a considerable amount of ROL and RE in lipid droplets**, although vitamin A can be delivered to adipose tissue as retinol (ROL) and retinyl esters (RE) and RA in the circulation (reviewed in [10]). WAT is the second largest storage site of vitamin A after the liver. ROL is the major form of RA precursors carried in the blood bound to the retinol-binding protein (RBP4)-transthyretin (TTR) complex (reviewed in [10, 118]). ROL uptake from the RBP4 complex is mediated by STRA6 receptor in WAT [119, 120]. ROL can also be delivered to the

cell when it is hydrolyzed from RE in chylomicron remnants. This uptake is mediated via lipoprotein lipase and occurs after hydrolysis of triglycerides from chylomicrons [121].

3. **RA is present in circulation at low nanomolar concentrations** [122]. Moreover, an endocrine role of naturally occurring RA is unknown. In blood, RA could be bound to lipocalins and/or albumin [123]. This RA could be from dietary origin and/or produced in peripheral tissues. An early study by Hill et al., showed that 15 min after oral intubation of mice with RA led to a predominant accumulation of RA in the small intestine, serum and liver [124]; however, RA is not stored in any tissue [123]. The contribution of circulating RA to effects in WAT or RA production in WAT has not been elucidated. Exogenously administered RA has marked effects on lipid metabolism and thermogenic adipose tissue remodeling (reviewed in [125, 126]); however, it remains unknown whether these functions could also be attributed to a physiological plasma pool of RA.
4. **RA regulates metabolism through post-transcriptional modification of proteins** [127] **in addition to major role of RA in activation of nuclear receptors** that is shared among all steroid and thyroid hormones [128]. RA isomers have high affinity to retinoic acid receptors (RAR α , RAR β and RAR γ) [129]. *9-cis* RA isomer and polyunsaturated fatty acids were identified as ligands for retinoid X receptors (RXR α , RXR β and RXR γ) (reviewed in [128]), but it is not known if *9-cis* RA is produced in WAT (Figure 5). Once RA is bound, RAR complexes with RXR [130]. This complex then binds to RA response elements (RAREs) in promoter/enhancer regions of target genes to regulate gene expression. The majority of nuclear receptors, including TR, vitamin D receptor, and PPAR γ require heterodimerization with RXR [129, 130]. Thus, through RXRs, RA can potentially influence whole body metabolism and immune response through partnership with other nuclear receptors. Dr. Noa Noy and co-workers showed that all-*trans* retinoic acid (atRA) can directly bind to PPAR δ at concentrations higher than 100 nM (K_d=770 nM) [131]. Because RA acts as a ligand to nuclear receptors, it can be considered a paracrine hormone. Indirectly, RA influences numerous other transcriptional pathways that play an important role in WAT (reviewed in [132]). These RA-dependent pathways include C/EBP transcription factors, STAT 5, and zinc finger protein 423 (Zfp423), which contribute to expression of a master regulator of adipogenesis PPAR γ [120, 133, 134] (reviewed in [132]).

RA binding to RAR receptor in cytosol has been linked to activation of mTOR kinase and translation in neurons [135]; however, the relevance of this and other pathways for adipogenesis has not been investigated. Cytosolic action of RA on signaling pathways plays a pivotal role in the commitment of stem cells and preadipocytes to adipogenesis. Prolonged treatment of embryonic stem cells with RA leads to activation of the extracellular-signal-regulated kinase (ERK) glycogen synthase kinase 3 (GSK) signaling via RAR β [136, 137]. These signaling cascades are required for PPAR γ expression and commitment of embryonic stem cells to adipogenesis. In contrast, in mesenchymal stem cells RA treatment leads to RAR β expression via Smad/p38MAPK and these signaling events stimulate adipogenesis.

The significance of signaling cascades as well as post-translational modifications of protein by RA for adipogenesis is less established compared to genomic effects of endogenous RA.

5. RA generation requires production of intermediate bioactive metabolites in WAT.

5.1. Retinaldehyde. RA production in WAT occurs in two enzymatic steps and includes production of an intermediate metabolite, retinaldehyde (Rald). Rald is known primarily for its role in the eye (reviewed in [138]), but in WAT, Rald regulates adipogenesis through inhibition of RXR and PPAR γ [139]. Rald also mediates non-genomic effects leading to rapid translation of ATGL, activation of lipolysis, and subsequent activation of thermogenesis in visceral WAT [9]. Direct molecular targets of Rald that mediate transcription and signaling have not been reported. The sequential production of RA from Rald and their opposing effects on regulation of PPAR γ expression and activities offer an opportunity for switching adipogenic to thermogenic processes in visceral adipocytes (reviewed in [140]).

5.2. Oxidized metabolites of RA. RA is metabolized by cytochrome P450 enzymes and its oxidized metabolites also activate RAR [141, 142]; however, the function of these RA derivatives in adipose tissue has not been reported.

6. **Enzymatic production of RA occurs in multiple steps that are controlled by multiple families of enzymes.** In WAT, ROL is hydrolyzed from RE stored in lipid droplets predominantly by hormone sensitive lipase (HSL) [143, 144]. The reverse conversion of ROL to RE is catalyzed by lecithin retinol acyltransferase (LRAT) [118]. In cytosol, ROL can be reversibly converted to Rald by short-chain reductases/dehydrogenases (SDR)/retinol dehydrogenase (RDH), the alcohol dehydrogenase (ADH), and aldo-ketoreductases families of enzymes (reviewed in [10]). Rald can be reduced to ROL by ADH and RDH families of enzymes or irreversibly oxidized to RA by the aldehyde dehydrogenase 1 (ALDH1) enzyme family (ALDH1A1, ALDH1A2, ALDH1A3) [reviewed in [132, 145]]. All ALDH1 enzymes are cytosolic NADPH-dependent aldehyde dehydrogenases [145]. Rald is the unique substrate of ALDH1A2 and ALDH1A3 enzymes and its conversion to RA is irreversible. Rald is a preferred substrate for ALDH1A1, however, this enzyme can contribute to oxidation of other aldehydes if they are present at high concentrations [146]. In different tissues, these enzymes have different patterns of expression [145] that could potentially control physiological production of Rald and/or RA in a tissue-specific manner.

7. **Endocrine, environmental, and dietary pathways are integrated in the expression pattern of Aldh1a1, Aldh1a2, and Aldh1a3.** ALDH1 family of enzymes showed sex-specific patterns of expression in WAT [9] as a result of dissimilar regulation of members of this family by sex hormones. The androgen response element in the *Aldh1a3* promoter induces expression of this gene in response to testosterone [147]. In contrast, estrogen suppresses *Aldh1a2* and *Aldh1a3* in mouse WAT [9]. Estrogen deprivation induces expression of *Aldh1a3* in ovariectomized mice [9]. Therefore, the main enzymes responsible for RA

generation in males and estrogen-deprived females are *Aldh1a1* and *Aldh1a3*, while in female WAT, *Aldh1a1* is the major RA-generating enzyme [9, 134]. During adipogenesis, *Aldh1a1* is responsible for approximately 70% of RARE activation [134].

The mechanisms responsible for ubiquitous expression of *Aldh1a1* in the majority of tissues [148] and for its predominant expression in WAT [9, 134] are the subject of intense investigations. *Aldh1a1* expression is under control of developmental and oncogenic transcription factors that control the cell cycle. *Aldh1a1* expression is repressed by homeobox protein HOX11/TLX1 [149]. Activation of *Aldh1a1* expression is mediated by cJun and its activator protein-1 (AP1) complex [150] and MUC1-C and C/EBP β complex [151]. Oxidative stress is another inducer of *Aldh1a1* expression. Nuclear factor E2-related factor 2 (NRF2) down-regulates *Aldh1a1* expression whereas environmental pollutants, such as acrolein, induces expression of this enzyme via AP1-dependent pathway [150]. *Aldh1a1* expression is induced during adipogenesis [134]; however, the relevance of developmental and oncogenic transcription factors for induction of *Aldh1a1* during adipogenesis and their participation in the formation of fat depots during embryogenesis has not yet been reported.

Compared to lean women, obese women express higher levels of *Aldh1a1* in the stromal vascular cell fraction isolated from omental adipose tissue [9]. This regulation could be caused by an increased content of cholesterol in obesogenic diets and/or oxidative stress associated with this diet. High-fat feeding is associated with induction of *Aldh1a1* and *Aldh1a2* expression via sterol regulatory element binding protein-1c (SREBP-1c) [152]. SREBP1c is expressed at higher levels in subcutaneous compared to omental visceral fat depots in women [153] suggesting that *Aldh1a1* and *Aldh1a2* expression levels will be higher in subcutaneous fat in response to the same high-fat diet. Indeed in mice, *Aldh1a1* and *Aldh1a2* were expressed at significantly higher levels in subcutaneous than in visceral adipose tissue [134]. In consonance, a high-fat diet markedly increases both obesity and RA-dependent RARE activation in WAT of RARE-lacZ transgenic mice that occurred in a sex and fat-depot-specific fashion [9]. The interruption of RA production in *Aldh1a1*^{-/-} females prevented formation of fat in the visceral depot in which RA production depends on *Aldh1a1* [9]. Thus, *Aldh1* gene regulation can integrate different hormonal, environmental, and dietary signals to produce RA in an adipose-depot specific fashion.

8. **Intracrine *Aldh1a1* circuits can control thermogenic reprogramming of WAT fat depots.** *Aldh1a1*^{-/-} mice have higher levels of Rald in WAT, resisted high-fat diet-induced obesity and insulin resistance, and had a higher metabolic rate and body temperature [139]. We found that resistance to high-fat diet was more pronounced in *Aldh1a1*^{-/-} female than *Aldh1a1*^{-/-} male mice as a result of the high rate of lipolysis and thermogenesis in the visceral fat depot, where ALDH1A1 was a single enzyme responsible for RA generation [9]. Although responses in *Aldh1a1*^{-/-} mice were a sum of systemic, tissue, and cell-based effects, thermogenesis was regulated largely autonomously, because immortalized

Aldh1a1^{-/-} fibroblasts maintained thermogenic characteristics in cell culture [23]. This study suggested that *Aldh1a1*-mediated intracrine signaling is sufficient to suppress thermogenesis in adipocytes. To examine if a subset of *Aldh1a1*^{-/-} thermocytes can reprogram thermogenesis in adipose depots of obese WT mice, we encapsulated *Aldh1a1*^{-/-} thermocytes into a biocompatible polymer that enable long-term cell survival in vivo [23]. We implanted encapsulated *Aldh1a1*^{-/-} thermocytes by injection into visceral fat depots of WT mice fed a high-fat diet for 80 days. The encapsulated *Aldh1a1*^{-/-} thermocytes induced marked thermogenic remodeling of surrounding host visceral WAT of WT mice [23] (Figure 6). This thermogenic remodeling was associated with the loss of lipid content in the adipose depot and an increase in systemic metabolic rate [23]. No thermogenesis was observed in non-implanted visceral WAT or visceral WAT implanted with WT preadipocytes expressing natural levels of *Aldh1a1*. The data suggest that interruption of intracrine ALDH1A1-dependent signaling in adipocytes can be an effective strategy for reduction of deleterious visceral fat. Thus, compared to other glandular pathways, RA production in adipose tissue appears to be a major contributor to the local pool of RA. Moreover, multi-enzyme pathways for generation of RA in different adipose depots offers more opportunities to achieve tissue-specific effects.

Expert opinion

Compared to administration of hormones, regulation of enzymes controlling hormone activation in tissues appears to be a better therapeutic strategy to decrease side effects. However, glandular production of steroid hormones and thyroid hormones regulates energy balance and, therefore, obesity in a systemic fashion that overpowers local hormone production. Although hormone-generating enzymes in adipose tissues play an important role in the formation of adipose depots, they also release hormones into circulation. Therefore, therapeutic regulation of these enzymatic pathways expects to elicit effects not only in fat depots, but also in other tissues where these hormones could become detrimental. ALDH1 and other vitamin A metabolizing enzymes exhibit specific expression patterns in tissues. The production of RA by multiple enzymes could be used as a unique opportunity to control RA production in a tissue-specific fashion. In female visceral fat, RA production is controlled by one ALDH1A1 enzyme. The interruption of this signaling in a subset of *Aldh1a1*^{-/-} adipocytes induces thermogenesis in these cells. Paracrine action of *Aldh1a1*^{-/-} adipocytes leads to thermogenic remodeling of the surrounding fat depot. The therapeutic potential of this strategy for targeted regulation of visceral obesity needs to be explored in the future.

Outlook

The future of medicine is in development of therapies free of side effects. It is achievable by tissue-specific treatments. Understanding para-/intracrine pathways in specific tissues, for example adipose depots, will provide important therapeutic targets for tissue-specific treatments. Another opportunity is often seen in applications of stem cells. However, in metabolic diseases, stem cell differentiation would be under control of already impaired

signaling. Delivery of genetically programmed cells for paracrine regulation of host tissue can potentially provide an effective solution for hormonal control in a specific tissue.

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Highlights

- Activation of steroid and thyroid hormones from prohormones occurs in glandular and extraglandular tissues.
- The pathway converting vitamin A into RA for regulation of WAT is unique.
- Enzymatic production of RA occurs in multiple steps that are controlled by multiple families of enzymes.
- WAT depots have a specific pattern of expression of RA-generating ALDH1 enzymes.
- Endocrine, environmental, and dietary pathways are integrated in the expression pattern of *Aldh1a1*, *Aldh1a2*, and *Aldh1a3*.
- Interruption of *Aldh1a1* circuits can control thermogenic reprogramming of WAT fat depots.
- Implantation of *Aldh1a1* deficient preadipocytes can induce thermogenesis in abdominal fat.

Figure 1.

Production of hormones (H) from prohormones (pro-H) and micronutrients in white adipose tissue (WAT) depots controls disparate formation of abdominal versus peripheral fat depots. Systemic obesity depends on systemic factors, such as diet, circulating hormones, and other circulating metabolites and cytokines that are produced in response to environmental and physiological stressors. Peripheral and abdominal fat depots express enzymes converting prohormones and micronutrients into hormones regulating nuclear receptors and other transcription factors (TF). This hormone-dependent regulation of TF can control fat-depot-specific gene expression and lead to dissimilar fat accumulation in abdominal and subcutaneous fat depots.

Figure 2.

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is a principal enzyme converting cortisone into cortisol in WAT. 11β-HSD1 predominantly catalyzes conversion of cortisone to cortisol, however, the reverse reaction can occur (arrow). Cortisol binding to the glucocorticoid (GR) or mineralcorticoid (MR) receptors lead to allosteric changes that can dislodge nuclear receptors from chaperons. GR can form homodimers or heterodimers with MR. MR can also dimerize with RXR. In the nucleus (dashed gray line), complex can bind to cognate response elements (MRE or GRE) in the regulatory sequences of target genes and initiate gene transcription. Activation of 11β-HSD1 induces abdominal fat formation, reactive oxygen species (ROS) and inflammation in this fat depot.

Figure 3.

Deiodinases regulate adaptive thermogenesis by conversion of thyroid hormones in white and brown adipose tissue.

T4 is transported from blood into the cytosol through an integrin transmembrane transporter. T3 and rT3 are transported from by monocarboxylate transporter 8 (MCT8). Deiodinases (D1, D2, D3) can convert T4 to T3 or rT3. Both T3 and rT3 can be catabolized to T2. T2 can elevate ATP synthesis and hydrolysis. It may the ability to reduce adiposity and dyslipidemia. T3 is a ligand for transcription factors thyroid receptors (TR α and TR β). T3 binding to TR leads to formation of TR:RXR heterodimer, recruitment of co-activators (CoA) and activation of target genes containing thyroid receptor response element (TRE). Nuclear activation of the TR by T3 results in increases in mitochondria biogenesis, respiratory enzymes transcription that assist adaptive thermogenesis. T3, T4 and non-classical thyroids also can affect mitochondrial respiration and sympathetic stimulation by non-genomic pathways involving phosphatidylinositol 3-kinase (PI3K). PI3K is presented as a regulatory subunit p58a and a catalytic subunit p110.

Figure 4.

Aromatase converts C19 steroids such as androstenedione (AN) and testosterone (TT) into C18 steroids estradiol (E2) and estrone (E1) to regulate activity of estrogen receptors (ERα and ERβ) and WAT depots.

Aromatase is encoded by Cyp19A1 gene. Depending on WAT depot, E2 preferentially activates ERα or ERβ target genes. Aromatase-dependent activation of ERβ in adipose tissue is associated with obesity and risks for breast cancer. Aromatase activity and CYP19A1 expression has been found to be up-regulated by GR agonists (dexamethasone and cortisol), cyclic adenosine monophosphate analogues (cAMP), TNFα, and prostaglandin E2 (PGE2). Also, higher expression of retinoic acid receptor alpha (RARα) is associated with a higher expression of CYP19A1 and a greater rate of aromatase activity. Flavonoids (Fl) and lignans (Ln) inhibit the activity of aromatase, preventing estrogen production. Flavonoids and lignans can also compete for the binding site of estrogen on estrogen receptors and inhibit E2-dependent gene transcription.

Figure 5.

Combined catalytic function of aldehyde dehydrogenase 1 family of enzymes (ALDH1A1, ALDH1A2, ALDH1A3) production of retinoic acid (RA) in WAT in a sex specific manner and fat depot-specific manner.

Expression of Aldh1 enzymes is different in males and females, which regulates dissimilar retinaldehyde (Rald) conversion to RA. Estrogen (E2) represses Aldh1a3 expression, whereas a high-fat (HF) diet increases *Aldh1a1* expression in women. RA has multiple genomic effects in adipose tissue. RA is a ligand for retinoic acid receptor (RAR) and regulates expression of numerous transcription factors. Aldh1a1-dependent production of RA induce expression of transcription factor *Zfp423* resulting in the expression of *Pparg*, a master regulator of adipocyte differentiation and fat formation. In the absence of *Aldh1a1*, and reduced expression of *Aldh1a2* and *Aldh1a3* in females, Rald is not converted to RA and increases protein levels of ATGL by an as yet unknown mechanism ('?'). Fatty acids that were hydrolyzed from triglycerides (TG) by ATGL, activate PPARα and its target gene Ucp1. This induction of thermogenesis occurs in visceral fat of *Aldh1a1*^{-/-} females, resulting in resistance to HF diet-induced abdominal obesity. Retinyl esters (RE) are carried by chylomicrons in the circulation and delivered to the cell where they are taken up by lipoprotein lipase (LPL) into lipid droplets. RE can be hydrolyzed by HSL into retinol (ROH). In the blood, ROH is bound in a retinol-binding protein (RBP4)-transthyretin (TTR) complex and is taken up by the STRA6 transporter into the cell. ROH can be converted to RE for storage in lipid droplets via lecithin retinol acyltransferase (LRAT) or it can be reversibly converted to rald by retinol dehydrogenase (RDH) or the alcohol dehydrogenase (ADH) family of enzymes. Rald can be irreversibly oxidized to RA by the ALDH1 enzyme family. RA serves as a negative feedback inhibitor of *Aldh1a1* expression. In the nucleus,

RA binds to RAR. 9cisRA isomer binds to RXR. Activated RXR can form a monomer or heterodimers with other activated nuclear receptors. The RAR:RXR heterodimer binds to retinoic acid response element on the DNA (RARE) and initiates transcription of target genes that affect the cell cycle, adipogenesis, thermogenesis, inflammation and apoptosis. Retinoids are carried intracellularly by binding proteins (not shown in this simplified schematics).

Figure 6.

Interruption of *Aldh1a1*-dependent RA production induces thermogenesis and reduces abdominal obesity in wild-type mice. *Aldh1a1*^{-/-} mice resisted abdominal obesity as a result of increased thermogenesis in abdominal WAT. Isolated and immortalized *Aldh1a1*^{-/-} preadipocytes (red arrows) have inheritable thermogenic properties. *Aldh1a1*^{-/-} preadipocytes could be used for transplantation into animals with different genetic backgrounds if they are encapsulated in a biocompatible porous nanomaterial for a long-term survival in the body. They are seen as large circular structures within host adipose tissue (red arrows). The implantation of encapsulated thermogenic *Aldh1a1*^{-/-} preadipocytes, but not wild-type adipocytes (green arrows), into abdominal WAT in obese wild-type female mice induced thermogenesis in the treated WAT depot. UCP1-positive brown staining indicates thermogenesis in the host tissue. This experiment demonstrates paracrine effects of *Aldh1a1*^{-/-} preadipocytes that could be used to induce thermogenic remodeling in abdominal fat without affecting other WAT depots.