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Short Article

Bone Marrow Adipose Tissue Is an Endocrine Organ that Contributes to Increased Circulating Adiponectin during Caloric Restriction

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SUMMARY

The adipocyte-derived hormone adiponectin promotes metabolic and cardiovascular health. Circulating adiponectin increases in lean states such as caloric restriction (CR), but the reasons for this paradox remain unclear. Unlike white adipose tissue (WAT), bone marrow adipose tissue (MAT) increases during CR, and both MAT and serum adiponectin increase in many other clinical conditions. Thus, we investigated whether MAT contributes to circulating adiponectin. We find that adiponectin secretion is greater from MAT than WAT. Notably, specific inhibition of MAT formation in mice results in decreased circulating adiponectin during CR despite unaltered adiponectin expression in WAT. Inhibiting MAT formation also alters skeletal muscle adaptation to CR, suggesting that MAT exerts systemic effects. Finally, we reveal that both MAT and serum adiponectin increase during cancer therapy in humans. These observations identify MAT as an endocrine organ that contributes significantly to increased serum adiponectin during CR and perhaps in other adverse states.

INTRODUCTION

White adipose tissue (WAT) is a major endocrine organ that exerts diverse systemic effects. One of the most extensively studied adipocyte-secreted factors is the hormone adiponectin, which promotes insulin sensitivity, fat oxidation, antiatherogenic, and anticancer effects (Ye and Scherer, 2013). Serum adiponectin is also a well-established biomarker for insulin resistance and cardiovascular disease; indeed, circulating adiponectin is low in obese, insulin-resistant individuals and other adverse metabolic states (Ye and Scherer, 2013). Conversely, serum adiponectin increases in lean, insulin-sensitive states such as with calorie restriction (CR) in animals and anorexia nervosa (AN) in humans (Combs et al., 2003; Dolezalova et al., 2007; Pannacciulli et al., 2003). Reduced circulating adiponectin in obesity most likely derives from decreased adiponectin expression and secretion, which may result from mitochondrial dysfunction or aberrantly increased inflammation, hypoxia, or endoplasmic reticulum stress (Ye and Scherer, 2013). Far less is known about why serum adiponectin increases in lean states. Although some studies report increased adiponectin expression in WAT during extensive CR (Qiao et al., 2011), most studies in mice and humans find that prolonged CR or extensive weight loss increases serum adiponectin without affecting adiponectin expression or secretion from WAT (Behre et al., 2007; Combs et al., 2003; Kovacova et al., 2009; Wang et al., 2006). Indeed, adiponectin expression in WAT decreases in human subjects with AN (Dolezalova et al., 2007). Adiponectin clearance is also unaltered during CR (Qiao et al., 2011). Thus, in lean states such as CR or AN, the paradoxical increase in serum adiponectin can occur without greater expression or secretion from WAT or decreased adiponectin clearance.

Our knowledge of adiponectin derives from extensive study of WAT biology over the past generation. In comparison, metabolic research has largely neglected another adipose depot, bone

Table 1. MAT, WAT, BMD, and Serum Adiponectin in Subjects with Anorexia Nervosa and Healthy Controls

	HC	AN	p Value
Age (years)*	27.8 ± 4.6	30.3 ± 6.8	0.46
Body mass (kg)	61.7 ± 6.1	49.0 ± 5.2	<0.0001
IBW percentage	100.4 ± 5.4	79.2 ± 4.9	<0.0001
BMI (kg/m^2)	22.3 ± 1.6	17.3 ± 1.3	<0.0001
Body fat percentage	27 ± 4.7	18.8 ± 5.5	<0.001
Total fat mass (kg)	17.1 ± 3.6	9.9 ± 3.2	<0.001
Total body BMD*	1.11 ± 0.10	0.97 ± 0.07	<0.001
L4 MAT*	0.53 ± 0.26	0.86 ± 0.36	<0.01
Metaphysis MAT	2.20 ± 0.87	3.93 ± 2.38	0.04
Diaphysis MAT	4.08 ± 2.76	6.76 ± 2.95	<0.02
Estimated MAT mass (kg)	2.16 ± 0.24	2.90 ± 0.21	<0.0001
MAT as a percentage of total fat mass	13.0 ± 2.7	31.5 ± 9.2	<0.0001
Total adiponectin (ng/ml)*	6,396 ± 1,864	9,573 ± 5,291	<0.03
HMW adiponectin (ng/ml)*	3,735 ± 1,476	6,406 ± 4,139	<0.01

Subjects with AN (n = 12) and HCs (n = 21) were characterized as described in the Experimental Procedures and Supplemental Information. Data are mean ±SD. Asterisks indicate that a Wilcoxon rank-sum test was used to compare these data because they were nonnormally distributed. IBW, ideal body weight.

marrow adipose tissue (MAT). Bone marrow (BM) adipocytes were identified over a century ago, and MAT accounts for approximately 70% of BM volume in adult humans (Fazeli et al., 2013). In striking contrast to WAT, MAT markedly increases during CR in animals, including humans (Devlin, 2011). Thus, in this manuscript, we investigate the hypothesis that MAT is a source of circulating adiponectin in states of leanness. We provide direct evidence that MAT is required for maximal increases in serum adiponectin during CR.

RESULTS

Anorexia Nervosa Is Associated with Increased Serum Adiponectin and MAT

Previous studies have not assessed both serum adiponectin and BM adiposity in a single cohort of AN subjects. Thus, we completed both analyses in a group of AN subjects and healthy controls (HC). Body mass index (BMI), adiposity and bone mineral density (BMD) were significantly lower in AN compared to HC subjects (Table 1). AN subjects had significantly higher MAT of the L4 vertebrae, femoral metaphysis and femoral diaphysis, and increased total and high molecular weight (HMW) serum adiponectin, despite decreased total fat mass (Table 1). Additional calculations revealed that MAT comprised 13.0% of total adipose mass in HC subjects and 31.5% in AN subjects. This striking increase strongly suggests that, during CR, MAT exists in an amount that has clear potential to exert systemic effects through secretion of endocrine factors such as adiponectin.

The Relative Expression and Secretion of Adiponectin Is Greater from MAT than from WAT

The ability of MAT to express or secrete adiponectin has not been addressed. To do so, we took advantage of the fact that, in mice, BM of lumbar vertebrae (LV) contains few adipocytes, whereas BM of caudal vertebrae (CV) is almost entirely MAT (Figure 1A). We found that adiponectin expression in CV of C57BL/6J mice was similar to that in inguinal WAT (iWAT), gonadal WAT (gWAT), and perirenal WAT (pWAT; Figure 1B). In contrast, expression of peroxisome proliferator-activated receptor γ (PPARγ) was similar between WAT and CV of some mice but much lower in CV of other mice. Other adipocyte proteins, such as fatty acid binding protein 4 (FABP4) and perilipin A, were also markedly decreased in CV in comparison to WAT, whereas hormone-sensitive lipase (HSL) expression was undetectable in CV (Figure 1B). Similar results were observed in C3H/HeJ mice (Figure S1A available online). However, because CV also contain many nonadipocyte populations, expression of adipocyte proteins in CV lysates might be diluted in comparison to that in WAT. Therefore, we next analyzed pure MAT from rabbit tibiae, in which there is a gradient of increasing adiposity from proximal to distal (Figure 1C). This pattern of MAT distribution, which is also typical of that in humans (Scheller and Rosen, 2014), allowed us to isolate intact pieces of pure MAT or red marrow (RM). No trabecular bone was observed in any of the MAT samples (Figure 1D). As in mice, adiponectin protein was robustly expressed in rabbit MAT relative to FABP4 and perilipin A (Figure 1E); this was also observed for *Adipoq* and *Fabp4* transcripts (Figure S1B). Expression of *Pparg* was similar between MAT and WAT, whereas *Cebpa* and *Lep* were lower in MAT (Figure S1B). These observations suggest that, in comparison to WAT, CV of mice and MAT of rabbits express adiponectin at disproportionately high levels relative to other typical adipocyte transcripts and proteins.

To investigate the ability of MAT to secrete adiponectin, we cultured rabbit MAT and WAT explants and analyzed adiponectin secretion into conditioned media. We found that MAT secreted adiponectin at levels far higher than WAT despite similar media total protein content (Figures 1F and S1C).

To test whether these observations extend to humans, we characterized WAT and MAT from three patients undergoing below-the-knee amputation. Unlike rabbit MAT, bone fragments were interspersed with MAT in two of the three samples (Figure 1G). Nevertheless, adiponectin expression was greater in MAT than WAT of these patients (Figure 1H). Strikingly, adiponectin secretion was also markedly higher from explants of MAT than WAT despite a similar degree of explant breakdown and total protein content of the conditioned media (Figures 1I and S1D). These results suggest that human tibial MAT has a greater capacity than WAT to both express and secrete adiponectin.

MAT Directly Contributes to Increased Serum Adiponectin during CR

The above results are consistent with our hypothesis that MAT is a source of circulating adiponectin. However, our observations in AN subjects are correlative; therefore, we next directly tested whether MAT expansion is required for increased serum adiponectin with CR. To do so, we used Ocn-Wnt10b mice, which overexpress Wnt10b from osteoblasts (Bennett et al., 2007). Wnt10b potently inhibits adipogenesis and causes high bone mass; hence, we hypothesized that Ocn-Wnt10b mice would resist MAT expansion with CR, allowing us to test whether

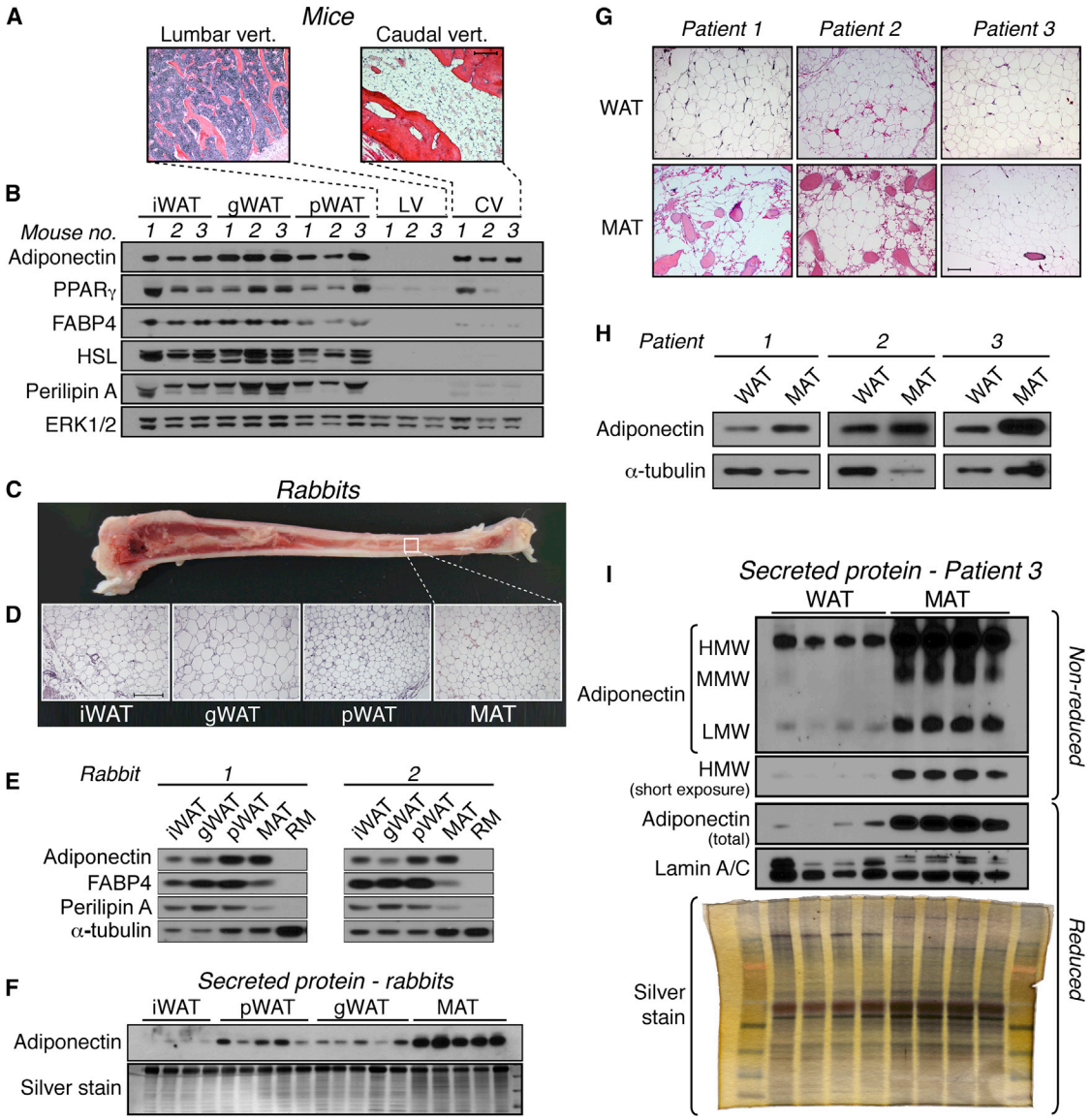

Figure 1. Relative Expression and Secretion of Adiponectin Is Greater from MAT than WAT

(A and B) iWAT, gWAT, pWAT, LV (for red marrow), and CV (for MAT) were isolated from male C57BL/6J mice.

(A) Micrographs of H&E-stained tissue sections.

(B) Immunoblots of total protein lysates from tissues of three mice. ERK1/ERK2 is a loading control. Phosphorylated forms of HSL and perilipin A appear as multiple bands.

(C–F) WAT, RM, and MAT were isolated from New Zealand white rabbits.

(C) Image of a bisected tibia showing the typical distribution of MAT.

(D) Micrographs of H&E-stained tissue sections.

(E) Immunoblots of total protein lysates from two rabbits, representative of four rabbits. α-tubulin is a loading control.

(F) Immunoblots and silver stain of conditioned media from WAT and MAT explants from one rabbit, representative of seven rabbits.

(G–I) Tibial MAT and scWAT were isolated from patients undergoing lower limb amputation.

(G) Micrographs of H&E-stained tissue sections.

(H) Immunoblots of total protein lysates of each tissue. α-tubulin is a loading control.

(I) Immunoblots and silver stain of conditioned media from explants of scWAT and MAT from patient 3. Lamins A and C were analyzed as an estimate of explant breakdown. Similar results were obtained for explants from patients 1 and 2 (Figure S1).

In (F) and (I), silver staining was used to assess total protein content. Images in (A)–(D) are representative of ten mice or rabbits. For micrographs, scale bars represent 200 μm. See also Figure S1.

increased MAT is required for elevated serum adiponectin in this context. We fed wild-type (WT) and Ocn-Wnt10b mice a control or 30% CR diet for 6 weeks. In female mice of both genotypes, CR significantly decreased body mass, blood glucose, lean mass, and liver mass but not fat mass (Figure S2A–S2C and 2A). Though not intuitive, this preservation of fat mass in female

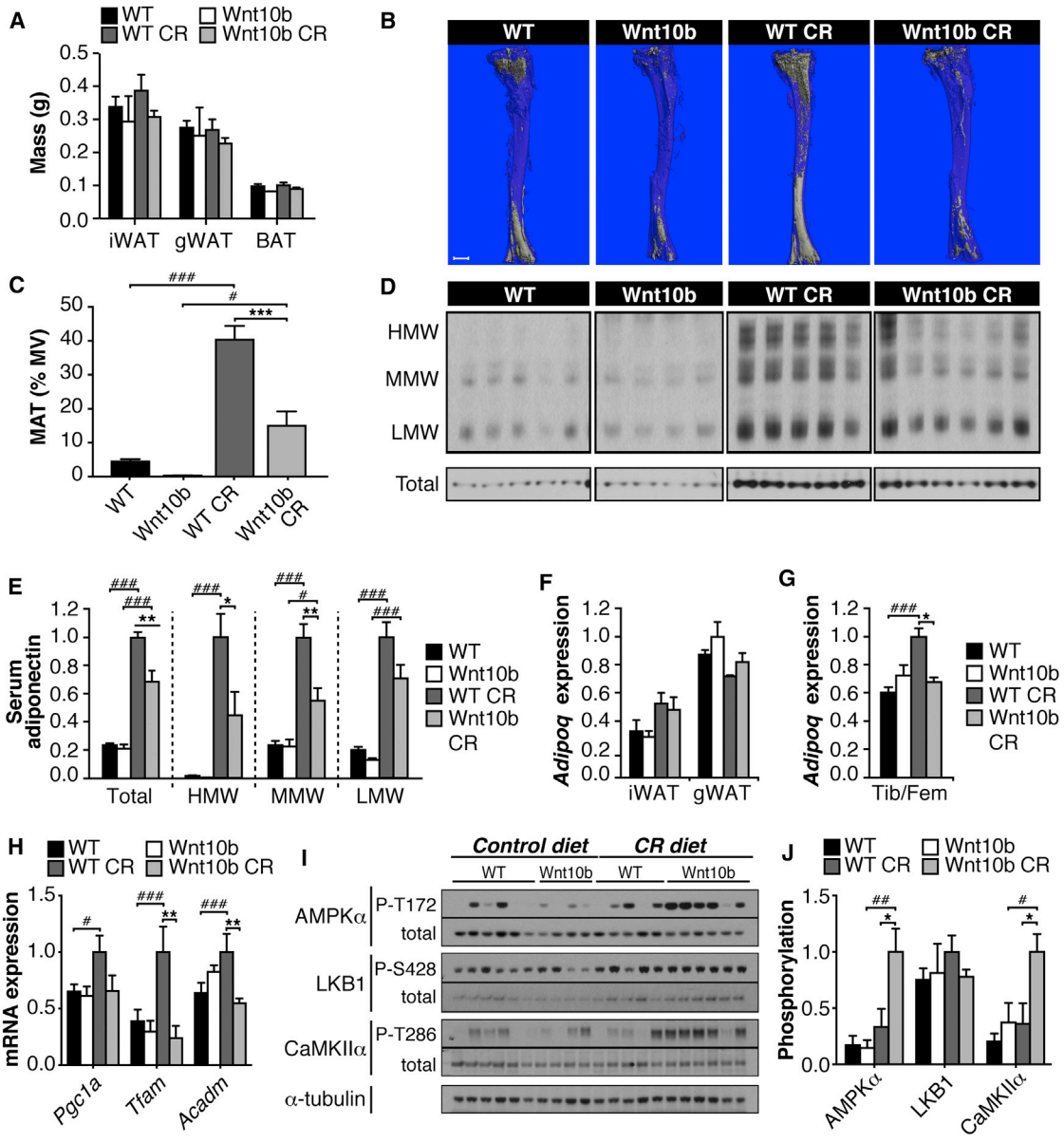

Figure 2. Blocking MAT Expansion Directly Prevents Increased Circulating Adiponectin during CR

WT and Ocn-Wnt10b mice were fed ad libitum or a 30% CR diet from 9–15 weeks of age.

(A) Masses of iWAT, gWAT, and BAT in 15-week-old mice.

(B and C) BM adiposity was assessed by osmium tetroxide staining of tibiae followed by μCT analysis.

(B) Representative images of stained tibiae. The scale bar represents 1 mm.

(C) MAT as a percentage of marrow volume (MV) was determined from μCT scans.

(D) Analysis of HMW, MMW, LMW, and total adiponectin in sera of 15-week-old mice. Immunoblots are from the same exposure of film.

(E) Quantitation of serum adiponectin from (D).

(F and G) qPCR analysis of *Adipoq* expression in iWAT, gWAT, or combined tibiae and femurs (Tib/Fem).

(H–J) Total RNA and protein was isolated from quadriceps muscle. Expression of *Pgc1a*, *Tfam*, and *Acadm* was determined by qPCR (H). Protein phosphorylation was determined by immunoblotting (I) and quantified by densitometry (J). CaMKIIα phosphorylation is a marker of Ca^{2+}/calmodulin signaling. Data are mean ±SEM of the following numbers of mice: WT, n = 6; Wnt10b, n = 4; WT CR, n = 5; and Wnt10b CR, n = 6. Similar results were observed in a second mouse cohort. For each diet group, significant differences between WT and Wnt10b mice are indicated by *p <0.05, **p <0.01, or ***p <0.001. Within each genotype, significant differences between ad libitum and CR diets are indicated by ##p <0.01 or ###p <0.001.

See also Figures S2 and S3 and Table S1.

C57BL/6J mice is consistent with previous CR studies (Li et al., 2010; Varady et al., 2010). Other expected effects of CR on WAT were also noted, including increased expression of fatty acid synthase protein (FAS) and transcripts (*Fasn*) (Figure S2D–S2F) (Bruss et al., 2010). Importantly, neither body mass nor fat mass differed between WT and Ocn-Wnt10b mice on either

diet. In contrast, Ocn-Wnt10b mice had markedly increased bone mass (Figure S2G and Table S1), consistent with our previous studies (Bennett et al., 2007). Next, we used osmium tetroxide staining to assess BM adiposity (Figures 2B and 2C). On a control diet, MAT did not significantly differ between WT and Ocn-Wnt10b mice. CR markedly increased MAT in WT mice; however, this increase was significantly blunted in Ocn-Wnt10b mice. Further analysis of whole tibiae and femurs revealed that CR increased expression of the adipocyte markers *Fabp4*, *Pparg*, and *Lep* in bones of WT mice but not in Ocn-Wnt10b mice (Figure S2H). Thus, both osmium tetroxide staining and quantitative PCR (qPCR) demonstrate that Ocn-Wnt10b mice resist MAT expansion during CR. Strikingly, CR-associated increases in serum adiponectin were also significantly blunted in Ocn-Wnt10b mice. HMW adiponectin was decreased by more than 50% in CR-fed Ocn-Wnt10b mice, and total, medium molecular weight (MMW), and low molecular weight (LMW) adiponectin were similarly decreased (Figures 2D and 2E). These differences are unlikely to be caused by differential hemoconcentration (Naruse et al., 2005), because silver staining revealed similar total serum protein content across all four groups (data not shown). We cannot exclude the possibility that adiponectin secretion from WAT is lower in Ocn-Wnt10b mice; however, WAT expression of *Gstk1*, *Erp44*, and *Erol1*, which encode regulators of adiponectin secretion (Ye and Scherer, 2013), was unaffected by CR or transgenic Wnt10b (Figure S2F; data not shown). These data are consistent with the finding that adiponectin secretion from WAT is unaltered during CR (Kovacova et al., 2009). Importantly, neither CR nor transgenic Wnt10b affected expression of *Adipoq* transcripts or protein in WAT (Figures 2F, S2D, and S2E). Thus, altered adiponectin expression in WAT does not account for the observed differences in serum adiponectin. In contrast, *Adipoq* expression in tibiae and femurs mirrored the changes in serum adiponectin (Figure 2G), suggesting that circulating adiponectin levels are directly related to adiponectin production from MAT. Therefore, these results provide direct evidence that MAT expansion is necessary for maximal production of serum adiponectin during CR.

Impaired MAT Expansion Alters Skeletal Muscle Adaptations to CR

Next, we investigated whether limiting the increases in MAT or adiponectin during CR has wider metabolic consequences. In obese states, lack of adiponectin causes glucose intolerance (Maeda et al., 2002; Ye and Scherer, 2013). However, glucose tolerance did not differ between WT and Ocn-Wnt10b mice (Figure S2I). This is consistent with adiponectin deficiency not affecting glucose tolerance in lean mice (Maeda et al., 2002). Expected effects of adiponectin or CR on liver transcript expression, including increased *Pparg*, *Ppara*, and *Hnf4a* and decreased *Fabp5* (Liu et al., 2012; Nogueira et al., 2012), also did not differ between WT and Ocn-Wnt10b mice (Figure S3). Therefore, we next analyzed skeletal muscle, a major metabolic target of adiponectin. Here, adiponectin stimulates Ca^{2+} influx and LKB1 activation, thereby enhancing AMPK activity, PPARγ coactivator 1α expression, and mitochondrial biogenesis (Ye and Scherer, 2013). Consistent with a previous report (Finley et al., 2012), in WT mice CR increased expression of *Pgc1a* and its downstream targets *Tfam* and *Acadm* (Figure 2H), which encode proteins with important mitochondrial functions. Strikingly, these effects of CR were absent in Ocn-Wnt10b mice (Figure 2H). In contrast, hepatic expression of *Pgc1a* and *Tfam* did not differ between genotypes (Figure S3), suggesting that consequences of impaired MAT expansion are specific to skeletal muscle. As reported elsewhere (Gonzalez et al., 2004), CR in WT mice did not affect steady-state AMPK activity in muscle (Figures 2I and 2J). Two pathways upstream of AMPK activation, LKB1, and Ca^{2+}/calmodulin signaling, were also unaffected by CR in WT mice (Figures 2I and 2J). In contrast, CR markedly activated AMPK and CaMKIIα in skeletal muscle of Ocn-Wnt10b mice without affecting LKB1 activity (Figures 2I and 2J). These observations demonstrate that blocking MAT expansion during CR alters local homeostatic adaptations in skeletal muscle.

Both MAT and Circulating Adiponectin Increase during Cancer Therapy in Humans

The above findings in humans, rabbits, and mice identify MAT as a key source of circulating adiponectin during CR. Next, we began to explore this relationship in other clinical conditions. BM adiposity increases during radiotherapy or chemotherapy for cancer (Georgiou et al., 2012); hence, we hypothesized that serum adiponectin might also increase during such treatments. To address this possibility, we analyzed MAT and serum adiponectin in patients undergoing therapy for ovarian or endometrial cancer. We found that both LV MAT and total serum adiponectin increased significantly at 6 and 12 months after onset of chemotherapy or radiotherapy, despite no change in total body fat (Figures 3A–3D). Thus, both MAT and serum adiponectin increase during cancer therapy without changes in total adiposity outside of the BM. This suggests that MAT might contribute to circulating adiponectin in contexts beyond CR.

DISCUSSION

Previous studies have investigated BM adipocytes as local regulators of skeletal remodeling (Scheller and Rosen, 2014); however, the present study identifies MAT as an endocrine organ that can exert systemic effects. We show that, in comparison to WAT, CV of mice and MAT of rabbits express adiponectin more highly than other adipocyte markers. Moreover, we reveal that secretion of adiponectin is far greater from MAT than from WAT of rabbits and humans. Our observations in AN subjects show that MAT can make up over 30% of total body fat, underscoring the notion that, during CR in humans, MAT exists in amounts sufficient to make a major contribution to circulating adiponectin. Conclusive evidence comes from our use of Ocn-Wnt10b mice as a model of specific MAT ablation. We show that, with CR, these mice resist increases in both MAT and serum adiponectin without differences in WAT mass or adiponectin expression in WAT. It should be noted that, in some contexts, CR can increase adiponectin expression in WAT (Qiao et al., 2011); however, our findings are consistent with previous studies (Behre et al., 2007; Combs et al., 2003; Dolezalova et al., 2007; Kovacova et al., 2009), which collectively suggest that elevated circulating adiponectin during CR can occur without increased adiponectin production from WAT. Notably, our results in

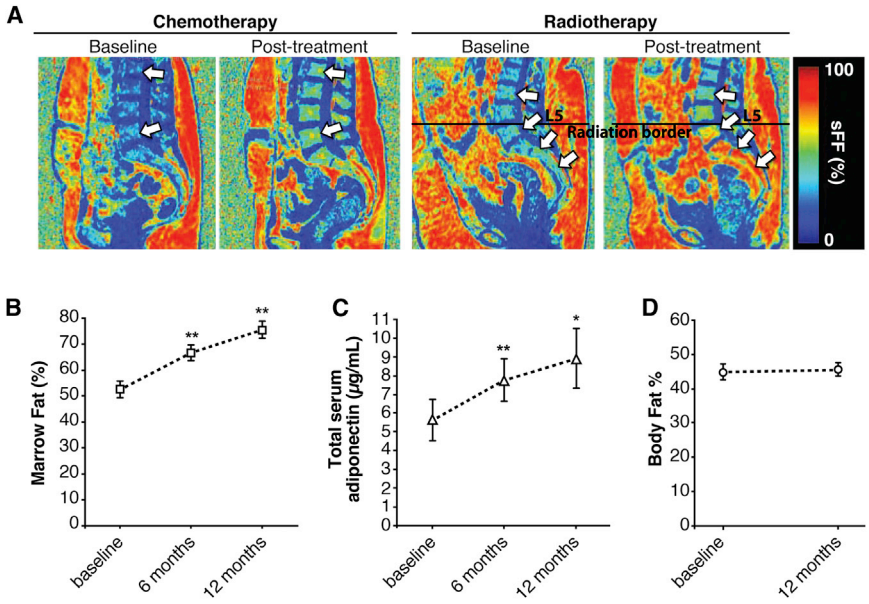

Figure 3. Both MAT and Serum Adiponectin Increase during Cancer Therapy in Humans

MAT, serum adiponectin, and body fat percentage were assessed in patients undergoing radiotherapy for endometrial cancer or chemotherapy for ovarian cancer.

(A) Representative MRI images of two patients before and at six months posttreatment. Arrows highlight increased vertebral MAT (signal fat fraction; sFF) posttreatment.

(B) MAT was determined by water-fat MRI at the indicated time points. Data are mean ±SEM of 11–15 patients.

(C) Total serum adiponectin concentrations were determined by ELISA and are mean ±SEM of 8–11 patients, with 11 patients assessed at baseline and 6 months, and eight of these patients also assessed at 12 months.

(D) Body fat percentage, as determined by DXA, shown as mean ±SEM of 11 patients.

For (B–D), statistically significant differences between baseline and 6 or 12 months posttreatment are indicated by *$p < 0.05$ or **$p < 0.01$.

Ocn-Wnt10b mice greatly extend these earlier studies by providing direct evidence that MAT is a key source of circulating adiponectin during CR. Thus, adiponectin production from MAT may account, at least in part, for the adiponectin paradox.

By producing adiponectin, MAT has the potential to exert systemic effects on metabolic homeostasis, immune responses, vascular function, or cancer risk. Indeed, we find that impaired MAT expansion during CR leads to altered metabolic adaptations in skeletal muscle, suggesting that MAT has effects beyond the skeleton. However, whether these effects are driven by changes in circulating adiponectin remains unclear. For example, the CR-associated increase in *Pgc1a*, *Tfam*, and *Acadm* in muscle of WT, but not Ocn-Wnt10b, mice, is consistent with the differences in circulating adiponectin, whereas increased AMPK and CaMKIIα activation with CR in Ocn-Wnt10b, but not WT mice, is highly unexpected. One possibility is that skeletal muscle of Ocn-Wnt10b mice adapts metabolically to a relative adiponectin deficiency by sustaining Ca^{2+}/calmodulin and AMPK activation. However, even in WT mice, skeletal muscle AMPK activity does not increase during CR (Figures 2I and 2J) (Gonzalez et al., 2004) despite increased circulating adiponectin. This suggests that, during CR, adiponectin does not stimulate AMPK in muscle. Indeed, existing knowledge of adiponectin action is largely limited to the context of obesity and insulin resistance (Ye and Scherer, 2013); the role of adiponectin during CR is poorly understood. In addition to adiponectin, we show that rabbit MAT expresses leptin at similar levels to iWAT and pWAT, and silver staining of human MAT- and WAT-conditioned media indicates that these tissues have distinct secretory profiles. These observations suggest that unique endocrine functions of MAT extend beyond adiponectin.

Finally, we reveal that both MAT and circulating adiponectin increase in patients undergoing cancer therapy. On the basis of these findings, it is tempting to speculate that the increased adiponectin derives from MAT expansion. Given that low circulating adiponectin is associated with increased cancer risk and that adiponectin can limit tumor growth (Dalamaga et al., 2012), the consequences of elevated adiponectin during cancer therapy clearly warrant further investigation. Beyond cancer, many other conditions are associated with increases in both MAT and circulating adiponectin, including aging, estrogen deficiency, type 1 diabetes, and treatment with pharmacologic agents such as thiazolidinediones or fibroblast growth factor 21 (Combs et al., 2003; Fazeli et al., 2013; Isobe et al., 2005; Kharitonenkov et al., 2007; Scheller and Rosen, 2014; Wei et al., 2012; Ye and Scherer, 2013). This suggests that MAT might impact upon circulating adiponectin in other clinically relevant conditions. In addition, both circulating adiponectin and MAT volume inversely correlate with BMD in human populations (Richards et al., 2007; Shen et al., 2012). This suggests that MAT might positively associate with circulating adiponectin in non-pathological contexts, as recently reported in Caucasian girls (Newton et al., 2013).

In summary, we have found that MAT expansion contributes significantly to increased serum adiponectin and skeletal muscle adaptation during CR. These findings suggest that MAT is a major source of circulating adiponectin in states of leanness and show that, through endocrine functions, MAT can act beyond the skeleton to exert systemic effects. However, the consequences of adiponectin production from MAT are yet to be fully established, and much about MAT biology remains unknown. Thus, future research in this area is clearly warranted if we are to better understand this understudied, clinically relevant tissue.

EXPERIMENTAL PROCEDURES

Additional procedures are described in the Supplemental Information.

Human Subjects

All work was performed as approved by the institutional review boards (IRBs) of the relevant institutions, as follows: AN study, Partners Human Research Office IRB; cancer therapy study, University of Minnesota IRB; human MAT studies, University of Michigan IRB.

Animals

All procedures were approved by the University of Michigan Committee on the Use and Care of Animals. C57BL/6J (000664) and C3H/HeJ (000659) mice were from the Jackson Laboratory. Ocn-Wnt10b mice (C57BL/6J background) were described previously (Bennett et al., 2007). Mice were housed on a 12 hr light/dark cycle in the Unit for Laboratory Animal Medicine at the University of Michigan with free access to water and, as indicated, food. Random-fed blood glucose, body fat, lean mass, and free fluid were assessed as described previously (Mori et al., 2012). For serum adiponectin, blood was taken from the tail vein of mice or the marginal ear artery of rabbits with Microvette CB300 capillary tubes (Sarstedt). Male New Zealand white rabbits, used at 11–18 weeks in age, were from Harlan Laboratories.

Caloric Restriction

Mice were fed a control diet (Research Diets, D12450B) or 30% CR diet (Research Diets, D10012703) from 9–15 weeks of age, as described previously (Devlin et al., 2010). Food was administered daily. The CR diet restricts macronutrient intake while maintaining mineral and vitamin levels. Mice were single housed from 8–9 weeks of age in order to determine average daily ad libitum food intake prior to CR.

Tissues

Tissues were fixed in 10% neutral-buffered formalin. Bones were decalcified in 14% EDTA for 14 days. Paraffin-embedded tissue sections were processed and stained with hematoxylin and eosin (H&E) or Toluidine blue, as indicated.

Statistical Analysis

Statistical analysis was performed with JMP 9.0 (SAS Institute), SPSS (IBM), or GraphPad Prism 6 (GraphPad Software), with means of normally distributed data compared with a two-tailed Student's t test and means of nonnormally distributed data compared with the Wilcoxon test. Significant differences in transcript expression of rabbit tissues were assessed with a paired Student's t test. Significant differences between WT and Ocn-Wnt10b mice were assessed with a two-sample Student's t test or ANOVA with post tests, as appropriate. Significant differences between MAT, serum adiponectin, and body fat percentage of human subjects undergoing cancer therapy were assessed with a Wilcoxon matched-pair signed rank test, with comparisons made between baseline and 6 or 12 months posttreatment. Error bars in the figures represent SEM. For all comparisons, a p value of < 0.05 was considered statistically significant.

SUPPLEMENTAL INFORMATION

Supplemental Information contains Supplemental Experimental Procedures, three figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2014.06.003.

AUTHOR CONTRIBUTIONS

W.P.C. and E.L.S. made equal contributions to the conceptualization, design, performance, and analysis of experiments and to the writing of the manuscript.

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