A-FABP mediates adaptive thermogenesis by promoting intracellular activation of thyroid hormones in brown adipocytes

Lingling Shu1,2,*, Ruby L.C. Hoo1,2,*, Xiaoping Wu1,2, Yong Pan1,2, Ida P.C. Lee1,2, Lai Yee Cheong1,3, Stefan R. Bornstein4, Xianglu Rong5, Jiao Guo5 & Aimin Xu1,2,3

The adipokine adipocyte fatty acid-binding protein (A-FABP) has been implicated in obesity-related cardio-metabolic complications. Here we show that A-FABP increases thermogenesis by promoting the conversion of T4 to T3 in brown adipocytes. We find that A-FABP levels are increased in both white (WAT) and brown (BAT) adipose tissues and the bloodstream in response to thermogenic stimuli. A-FABP knockout mice have reduced thermogenesis and whole-body energy expenditure after cold stress or after feeding a high-fat diet, which can be reversed by infusion of recombinant A-FABP. Mechanistically, A-FABP induces the expression of type-II iodothyronine deiodinase in BAT via inhibition of the nuclear receptor liver X receptor α, thereby leading to the conversion of thyroid hormone from its inactive form T4 to active T3. The thermogenic responses to T4 are abrogated in A-FABP KO mice, but enhanced by A-FABP. Thus, A-FABP acts as a physiological stimulator of BAT-mediated adaptive thermogenesis.
Obesity, which is caused by an extended energetic imbalance between energy intake and energy expenditure, is an important risk factor for type 2 diabetes and cardiovascular diseases\(^3\). Although the main function of white adipose tissue (WAT) is to store excess energy, brown adipose tissue (BAT), which is characterized by multi-locular oil vacuoles, high mitochondrial content and the presence of unique mitochondrial inner membrane protein uncoupling protein-1 (UCP-1), dissipates energy as heat\(^2\). Thus, BAT-mediated adaptive thermogenesis can be considered a defense mechanism that protects the organism against hypothermia or excessive weight gain in response to low temperature, or excess nutrient supply\(^5\). Accumulating evidence suggests that enhancing organismal thermogenesis is a promising therapy to combat obesity\(^4\).

Adaptive thermogenesis is primarily regulated by sympathetic nervous system (SNS), which heavily innervates interscapular BAT\(^5\). When the SNS is activated, catecholamines are released from sympathetic nerve endings and activate \(\beta\)-adrenergceptors on adipocytes, leading to an increase of intracellular cyclic adenosine monophosphate (cAMP) level. Elevated cAMP activates protein kinase A (PKA), which induces UCP-1 expression and activity\(^5\). Activated PKA also promotes lipolysis via stimulating hormone sensitive lipase (HSL) to provide free fatty acids (FFAs) as energy substrate for \(\beta\)-oxidation in mitochondria and UCP-1 expression\(^5\).

Thyroid hormones also contribute to adaptive thermogenesis in BAT by coordinating with SNS to induce expression of thermogenic genes\(^6\). Intracellular conversion of thyroxine (T4) into bioactive 3,3,5-triiodothyronine (T3) by type II iodothyronine deiodinase (D2) is required to activate the transcriptional program of thermogenic genes\(^7\). Other hormones, produced by various tissues, that have also been shown to induce BAT activity or browning of WAT include natriuretic peptides\(^8\), produced by various tissues, that have also been shown to induce in adipocytes\(^16\), but also produced in macrophages\(^17\), endothelial (IL4, IL13 and IL33) (refs 13–15).

The adipokine adipocyte fatty acid-binding protein (A-FABP, also known as FABP4 or aP2) is abundantly expressed in adipocytes\(^16\), but also produced in macrophages\(^17\), endothelial cells\(^18\) and glial cells\(^19\). A-FABP functions as a lipid chaperone that regulates trafficking, fluxes and signalling of FFAs, and has an important role in linking lipid metabolism with inflammation\(^20\). Although A-FABP was originally identified as an abundant cytoplasmic protein in adipocytes, a portion of A-FABP is released into bloodstream and acts as a humoral factor to regulate glucose and lipid metabolism\(^21,22\). Circulating A-FABP is elevated in obese individuals and correlates positively with the features of the metabolic syndrome, and the incidence of atherosclerosis and cardiovascular diseases\(^18\). Interestingly, A-FABP knockout (KO) mice are protected against high-fat diet (HFD)-induced metabolic dysfunction but exhibit increased adiposity compared with their wild-type (WT) littermates\(^23\). RNAi-mediated germline knockdown of A-FABP leading to a partial loss of A-FABP in mice also increases the susceptibility to diet-induced obesity\(^24\). Elevated A-FABP expression is observed in BAT of hibernating animals and cold-induced rodents\(^25,26\). Expression of A-FABP messenger RNA (mRNA) is also increased together with other thermogenic genes in BAT and WAT of HFD-induced UCP-1 deficient mice, suggesting that A-FABP might mediate a compensatory mechanism to maintain energy homeostasis\(^27\). A recent study demonstrated that ablation of both A-FABP and epidermal-FABP (E-FABP) impairs adaptive thermogenesis in mice in response to fasting and cold stress\(^28\). However, the underlying mechanism whereby A-FABP regulates energy metabolism remains elusive.

In this study, we found that the obese A-FABP KO mice have a marked attenuation of both HFD- and cold-induced BAT activation and energy expenditure, and this phenotype could be reversed by replenishment of recombinant A-FABP (rA-FABP). Mechanistically, we uncovered a role of A-FABP in promoting the intracellular conversion of T4 to T3 in BAT, and show that this is mediated in part by facilitating the transport of circulating FFAs released from WAT to BAT, which in turn enhances thermogenesis.

Results

A-FABP KO mice are defective in adaptive thermogenesis. To explore the potential roles of A-FABP in the regulation of energy metabolism, global A-FABP knockout (KO) mice were generated\(^29\) and fed with standard chow (STC) or HFD. A-FABP KO mice are more susceptible to diet-induced obesity as compared with their WT littermates (Fig. 1a). After feeding with HFD for 24 weeks, body weight of WT mice was significantly increased by 43.3 \(\pm\) 2% and the body weight gain in A-FABP KO mice was even more drastic (\(\sim 106.7 \pm 2.5\%\)) when compared with their respective STC-fed controls (Supplementary Fig. 1a). Body composition analysis showed no obvious difference in either lean mass or body fluid between A-FABP KO mice and WT controls on either STC or HFD. By contrast, the fat mass in HFD-fed A-FABP KO mice was 1.6-folds higher than the WT littermates (Supplementary Fig. 1b). This was further confirmed by dissection of mice showing a significant expansion in most of the fat pads in HFD-induced KO mice when compared with the respective WT controls (Supplementary Fig. 1c). No significant difference was observed in the weight of internal organs between WT or A-FABP KO mice. The weight of liver was even lighter in A-FABP KO mice (Supplementary Fig. 1d). Notably, there was no difference in the calorie intake between WT and A-FABP KO mice when fed with either STC or HFD (Supplementary Fig. 1e). Consistent with the previous study\(^23\), A-FABP KO mice exhibited an improved metabolic profile as indicated by the significant alleviation of HFD-induced glucose intolerance and insulin resistance (Supplementary Fig. 1f–h), a markedly reduced serum insulin and glucose levels, lipid profiles and a significantly higher adiponectin level compared with WT controls (Supplementary Fig. 1i–m).

Since A-FABP deficiency does not affect calorie intake, we next investigated whether HFD-induced morbid obesity in A-FABP KO mice was attributed to attenuated energy expenditure. The whole-body oxygen consumption (VO\(_2\)) was comparable between STC-fed WT and A-FABP KO mice (Fig. 1b), while A-FABP KO mice fed with HFD for 4 weeks displayed a significantly lower oxygen consumption compared with the relative WT controls (Fig. 1c). HFD-induced A-FABP KO mice displayed a higher respiratory exchange ratio (Fig. 1d). In addition, glucose uptake in soleus muscle and liver was also significantly increased in A-FABP KO mice when compared with their WT littermates (Supplementary Fig. 1n), suggesting that A-FABP KO mice prefer to utilize carbohydrate rather than fatty acid as energy substrate. There was no obvious difference in locomotory activity (Fig. 1e) between HFD-induced WT and A-FABP KO mice.

To further verify the role of A-FABP in adaptive thermogenesis, HFD-fed WT and KO mice were housed at 6\(^\circ\)C for 8 h. Rectal temperature was dropped from \(\sim 37^\circ\)C to 34\(^\circ\)C in the first 4 h in both the groups. Afterwards, the rectal temperature of A-FABP KO mice maintained at \(\sim 34^\circ\)C while that of WT littermates gradually increased and significantly higher than that of A-FABP KO mice after prolonged cold exposure (Fig. 1f). Furthermore, A-FABP KO mice exhibited a significantly less fat loss compared with WT controls after cold exposure for
Data are represented as mean ± s.e.m. (*P < 0.05, **P < 0.01, ***P < 0.001 (Student’s t-test)).

8 h (Fig. 1g). Taken together, these findings suggest that the morbidly obese phenotype of HFD-induced A-FABP KO mice is attributed to the impaired adaptive thermogenesis.

A-FABP deficiency impairs BAT recruitment in mice. Brown adipose tissue (BAT) is the major organ responsible for adaptive thermogenesis. We next examined the impact of A-FABP deficiency in BAT recruitment in response to HFD and cold exposure. STC-fed A-FABP KO and WT mice showed comparable BAT morphology, while HFD-fed A-FABP KO mice displayed larger lipid droplets but reduced multi-locular structures compared with WT controls (Fig. 2a, top). When exposed to 6 °C for 24 h, size and number of lipid droplets in HFD-fed WT mice were decreased more apparently than those in A-FABP KO mice (Fig. 2a, bottom). This was confirmed by measurement of triglyceride levels in BAT (Fig. 2b).

HFD or cold exposure induced approximately a fourfold increase of UCP-1 expression in BAT of WT mice, whereas A-FABP deficiency significantly attenuated cold- and HFD-induced expression of UCP-1 (Fig. 2c–e). Consistently, in response to HFD or cold challenge, A-FABP KO mice exhibited compromised induction in the expression of thermogenic genes (PGC-1α and Cidea) (Fig. 2f,g) while the expression of membrane fatty acid transporters such as CD36 and fatty acid transporter protein 1 were induced to a similar levels in both types of mice (Supplementary Fig. 2). Notably, A-FABP KO mice exhibited a similar cold-induced expression of UCP-1 and thermogenic genes in the subcutaneous fat as that in the WT littermates (Supplementary Fig. 3).

A-FABP facilitates the transportation of FFAs into BAT. A-FABP is not only a cytoplasmic protein, but also present in the circulation. We next investigated the roles of circulating A-FABP in thermogenesis. Circulating A-FABP in C57BL/6N mice was progressively elevated upon feeding with HFD, and this change was accompanied by increased level of FFAs (Fig. 3a,b). When C57BL/6N mice were subjected to acute cold exposure, both circulating A-FABP and FFAs were increased to a peak level in 1 h, and gradually declined to a basal level (Fig. 3c,d). Similar results were observed in mice treated with the β-adrenergic receptor agonist norepinephrine (Fig. 3e,f), suggesting that circulating A-FABP may be released simultaneously with FFAs upon thermogenic stimulation. Notably, A-FABP abundance also increased markedly in WAT of C57BL/6N mice in response to HFD or cold exposure (Supplementary Fig. 4), suggesting that WAT may be the main source of elevated circulating A-FABP and FFAs. Therefore, we next tested whether A-FABP promotes adaptive thermogenesis by facilitating the transport of FFAs into BAT.

Infusion of [3H]-palmitate in C57BL/6N mice followed by co-immunoprecipitation with anti-A-FABP antibody revealed that serum A-FABP could form complex with circulating
In vivo radioisotopic tracing showed that uptake of $^3$H-palmitate in BAT and WAT of A-FABP KO mice were significantly lower than that in their WT littermates (Fig. 3h) while replenishment with rA-FABP, but not its mutant R126Q which does not have binding capacity to FFAs\textsuperscript{30}, significantly enhanced the uptake of $^3$H-palmitate in BAT and WAT (to a much lower extent) in both WT and A-FABP KO mice (Fig. 3h,i). This was further confirmed by the result of in vivo BODIPY-FA fluorescence chasing experiment (Supplementary Fig. 5a,b). Similarly, BODIPY-FA uptake was markedly attenuated in A-FABP-deficient primary brown adipocytes, while pre-incubation of BODIPY-FA with rA-FABP enhanced uptake of FFAs in both A-FABP deficient- and WT adipocytes (Fig. 3j). Furthermore, rA-FABP exhibited a higher efficiency than the classical FFA carrier bovine serum albumin (BSA) in promoting FFA uptake in A-FABP-deficient adipocytes (Fig. 3k). Fluorescent-labelled rA-FABP entered adipocytes together with BODIPY-FA simultaneously while no fluorescent signal was detected in the control in which no rA-FABP was added (Fig. 3l). Replenishment of rA-FABP but not its mutant R126Q significantly increased the FFA level in BAT and this change was accompanied by a decreased FFA level in

**Figure 2 | A-FABP deficiency impedes HFD- and cold-induced activation of BAT in mice.** Male 4-week-old A-FABP KO and their WT littermates were fed with either STC or HFD for 24 weeks and subjected to room temperature (23 °C) or cold exposure (6 °C) for 24 h. (a) Haematoxylin and eosin (H&E) staining, (b) triglyceride levels, (c) immunohistochemistry (IHC) staining and densitometry analysis (right panel) for UCP-1 in brown adipose tissue (BAT) of mice. Scale bar, 20 μm, with magnification of 400 ×. Representative images from three independent experiments are shown (n = 8). (d,e) BAT isolated from above mice (d) fed with STC or HFD for 24 weeks or (e) exposed to 23 °C or 6 °C for 24 h were subjected to immunoblotting using an antibody against UCP-1, β-tubulin as indicated. Right panels are the band intensity of UCP-1 relative to β-tubulin and expressed as arbitrary units (n = 8). (f,g) The mRNA abundance of the thermogenic genes in BAT of above mice (f) fed with STC or HFD for 24 weeks or (g) exposed to 23 °C or 6 °C for 24 h (n = 8). Uncropped western blot images are shown in Supplementary Fig. 13. Data are represented as mean ± s.e.m. *P < 0.05, **P < 0.01 (one-way analysis of variance with Bonferroni correction for multiple comparisons).
WAT (Supplementary Fig. 5c) implicating that A-FABP acts as a FFA chaperone in transporting FFAs released from WAT to BAT. Notably, a significant portion of fluorescent-labelled rA-FABP administered through tail vein injection was delivered to BAT and WAT (to a lower extent). Cold exposure further enhanced the accumulation of rA-FABP in the BAT (Supplementary Fig. 5d,e). Furthermore, pre-incubation of palmitate with rA-FABP enhanced palmitate-induced oxygen consumption rate (OCR) in A-FABP-deficient adipocytes (Fig. 3m). Taken together, these data suggest that exogenous A-FABP facilitates the uptake of circulating FFAs into brown adipocytes to enhance its utilization.

**A-FABP enhances energy expenditure in A-FABP KO mice.**
To further determine whether circulating A-FABP promotes adaptive thermogenesis, rA-FABP or its mutant R126Q was continuously delivered into the circulation of 4-week HFD-fed A-FABP KO mice for a period of 2 weeks during which the whole-body energy expenditure was measured. rA-FABP and
its mutant R126Q were detectable in a comparable level in the circulation (Fig. 4a), BAT and WAT (Supplementary Fig. 6a,b). The whole-body energy expenditure was significantly increased by 1.5-folds in rA-FABP-treated A-FABP KO mice compared with PBS-treated mice (Fig. 4b,c). The A-FABP KO mice infused with the mutant R126Q also showed an increase of oxygen consumption, but only to an approximately half extent as that of mice infused with rA-FABP (Fig. 4b,c). Notably, infusion of rA-FABP or R126Q caused a modest decrease in body weight and fat mass, whereas the calorie intake was not affected (Supplementary Fig. 6d,c).

To evaluate the effect of A-FABP in modulating BAT recruitment, A-FABP KO mice infused with the above proteins were subjected to 6°C for 8 h. rA-FABP significantly increased the body temperature of A-FABP KO mice (Fig. 4d), suggesting an improved cold tolerance. However, this effect on cold tolerance was significantly attenuated in mutant R126Q-infused A-FABP KO mice (Fig. 4d). There was a remarkable increase of cold-induced multi-locular structures and upregulated UCP-1 expression in BAT of rA-FABP-infused KO mice (Fig. 4e,f), and were accompanied by a significantly elevated expression of thyroidogenic genes (PGC-1α, Cidea and Dia2) (Fig. 4g) comparing to the PBS-infused control mice. However, the potency of the mutant R126Q in inducing the expression of UCP-1 and other thermogenic genes (except Dio2) were significantly lower than rA-FABP (Fig. 4f,g). Consistently, infusion with rA-FABP or R126Q to A-FABP KO and WT mice also modestly enhanced BAT recruitment at 23°C but such an effect was much lower than their corresponding mice at 6°C (Supplementary Fig. 6e–g and Supplementary Fig. 7). Notably, there was no obvious change in glucose tolerance, insulin levels and insulin sensitivity (as determined by the insulin resistance index) in these recombinant proteins-infused mice comparing to the PBS-infused mice (Supplementary Fig. 8a–c,f–h). The inflammatory status in the peripheral tissues was not altered in these mice (Supplementary Fig. 8d,e,i,j). Taken together, these data suggest that the fatty acid binding capacity of A-FABP is at least partially contributed to its ability for the enhancement of thermogenesis. However, the mutant R126Q was unable to increase FFA uptake while it could still partially reverse the impairment of adaptive thermogenesis in A-FABP KO mice, indicating that there may be an additional mechanism contributing to the ability of A-FABP in promoting thermogenesis.

A-FABP deficiency impairs conversion of T4 to T3 in BAT. To explore additional mechanism whereby A-FABP regulates adaptive thermogenesis, we investigated whether A-FABP deficiency altered SNS activity. Oxygen consumption of A-FABP KO and WT mice on STC or HFD were comparable in response to norepinephrine (Supplementary Fig. 9a,b). Likewise, norepinephrine-induced circulating FFA levels were similar between HFD-induced WT and A-FABP KO mice (Supplementary Fig. 9c). HFD-induced expression of β adrenergic receptor 3 (ADRB3) and the activation of tyrosine hydroxylase in BAT were significantly increased to a similar level in both types of mice (Supplementary Fig. 9d,e). These data suggest that A-FABP deficiency does not attenuate SNS activity and lipolytic machinery in mice.

Since SNS and thyroid hormones regulate adaptive thermogenesis cooperatively, we evaluated whether A-FABP deficiency impedes the activation of thyroid hormones. There was no difference in circulating T4 or T3 levels between A-FABP KO mice and their WT littermates fed with either STC or HFD (Fig. 5a,b). T4 is the major form of thyroid hormone in blood while it has to be converted to the activated form T3 within its target tissues. T3 levels in BAT of WT mice were increased significantly upon HFD feeding or cold exposure while this induction was abrogated in A-FABP-deficient BAT (Fig. 5c,d). To investigate the effect of A-FABP in intracellular conversion of thyroid hormones, HFD-induced A-FABP KO and WT mice were supplemented with PBS or T4 for 5 consecutive days followed by cold exposure for 24 h. Supplementation of T4 enhanced cold-induced energy expenditure in WT mice, whereas such an effect of T4 administration was significantly attenuated in A-FABP KO mice (Fig. 5e). However, energy expenditure induced by supplementation of T3 was comparable between A-FABP KO and WT mice (Fig. 5f), implicating that A-FABP deficiency abolished T4 to T3 conversion in BAT. Multi-locular structures and elevated UCP-1 expression were observed in BAT of WT mice treated with either T4 or T3 (Fig. 5g,h). However, treatment with T3, but not T4, induced such changes in A-FABP KO mice (Fig. 5g,h). Similar results were observed in T4- or T3-treated WT and A-FABP KO mice under 23°C (Supplementary Fig. 10a–d), although the magnitude of these changes was much smaller compared with those at 6°C. The body weight, body composition and calorie intake were not altered in both types of mice under these circumstances (Supplementary Fig. 10e–h). Consistently, systemic supplementation with T3 markedly increased T3 levels in BAT of both genotypes (Fig. 5i), while treatment with T4 could only increase T3 levels in BAT of WT mice but not in A-FABP KO mice (Fig. 5j), suggesting that A-FABP is required for conversion of T4 to T3 within BAT.

**Figure 3 | Circulating A-FABP facilitates the uptake of free fatty acid into adipocytes.** (a) Circulating A-FABP and (b) FFA profile of male 4-week-old C57BL/6N mice fed with HFD for 24 weeks (n = 8). (c) Circulating A-FABP and (d) FFA profile of male 8-week-old C57BL/6N mice during cold exposure (6°C) for 4 h (n = 8). (e) Circulating A-FABP and (f) FFA level of male 8-week-old C57BL/6N mice intraperitoneally injected with norepinephrine (NE; 1 mg kg⁻¹) or PBS (vehicle) for 4 h under fasting condition (n = 6). (g) Co-immunoprecipitation (Co-IP) of A-FABP and 3H-palmitate in serum of male 8-week-old C57BL/6N mice after administration of 3H-palmitate (2 μCi) for 4 h. Right panel is the 3H-palmitate radioactivity of the co-immunoprecipitated A-FABP protein (n = 6). (h) 3H-palmitate uptake in BAT and WAT of 8-week-old A-FABP KO mice and their WT littermates infused with PBS or recombinant A-FABP (rA-FABP; 1 μg h⁻¹) or mutant R126Q (1 μg h⁻¹) (n = 6). (i) BODIPY-FA uptake in WT or A-FABP-deficient brown adipocytes treated with PBS or rA-FABP (2 μg ml⁻¹) for 10 min (min) (n = 6). (k) 3H-palmitate uptake in A-FABP-deficient adipocytes incubated with PBS, bovine serum albumin (BSA; 3 μg ml⁻¹) or rA-FABP (2 μg ml⁻¹) (n = 6). (l) In vitro fluorescent imaging analysis of brown adipocytes treated with BODIPY-FA (2 μM) with or without pre-incubation with fluorescence-labelled rA-FABP (2 μg ml⁻¹). Images were taken at 5, 10 and 30 min after treatment. Control image was taken at 30 min in which A-FABP-deficient brown adipocytes were incubated with BODIPY-FA without pre-incubation with rA-FABP. Scale bar, 20 μm, with magnification of 400 x. Representative images from three independent experiments are shown (n = 6). (m) Oxygen consumption rate (OCR) and its mean value (lower panel) of A-FABP-deficient brown adipocytes treated with palmitate (PA: 200 nM) with or without pre-incubation with rA-FABP (2 μg ml⁻¹) (n = 6). CPMA, count per minutes for beta particles; RFU, relative fluorescence units; OCR, oxygen consumption rate; FCCP, carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone; R/A, rotenone/antimycin A. Uncropped image for co-immunoprecipitation is shown in Supplementary Fig. 13. Data are represented as mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001 (Student’s t-test (a–g), one-way analysis of variance with Bonferroni correction for multiple comparisons (h–k,m)).
**A-FABP enhances T3 levels in BAT via LXRα-Dio2 pathway.**

Type II iodothyronine deiodinase (D2), which is encoded by the Dio2 gene, is the key enzyme responsible for local T4 to T3 conversion. Dio2 is downregulated by the nuclear receptor LXRα, while A-FABP represses LXRα activity and expression in macrophages. Therefore, we investigated whether A-FABP enhances T3 levels in BAT via LXRα-Dio2 pathway.

**Figure 4 | A-FABP enhances energy expenditure and BAT recruitment in A-FABP KO mice.** Male 4-week-old A-FABP KO mice fed with HFD for 4 weeks were infused with PBS (vehicle), recombinant A-FABP (rA-FABP, 1 μg h⁻¹) or A-FABP mutant R126Q (1 μg h⁻¹) for 14 days with or without subjected to cold exposure (6 °C). (a) Circulating rA-FABP level and (b) oxygen consumption (VO₂) of A-FABP KO mice before or after infusion of recombinant proteins (n = 6). (c) Mean VO₂ of above A-FABP KO mice measured after infusion of recombinant proteins for 3 days (n = 6). (d) Rectal temperature of above A-FABP KO mice infused with rA-FABP or R126Q during cold exposure (6 °C) for 8 h. (e) Haematoxylin and eosin staining and IHC staining of UCP-1 in BAT of mice after cold exposure for 8 h, scale bar, 20 μm; with magnification of 400 x. The right panel is the densitometry analysis for UCP-1. Representative images from three independent experiments are shown (n = 6). (f) BAT isolated from above mice was subjected to immunoblotting using an antibody against UCP-1, β-tubulin as indicated. The right panel is the band intensity of UCP-1 relative to β-tubulin (n = 6). (g) The mRNA abundance of the thermogenic genes PGC-1α, Cidea and Dio2 in BAT isolated from above mice (n = 6). CPMA, count per minutes for beta particles. N.D., not detected.

Uncropped western blot images are shown in Supplementary Fig. 13. Data are represented as mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, $rA-FABP$ versus R126Q, $S < 0.05; #R126Q$ versus PBS, $#P < 0.05$ (One-way analysis of variance with Bonferroni correction for multiple comparisons).
regulates the conversion of T4 to T3 via LXRα-Dio2 signalling pathway. HFD and cold exposure greatly induced the expression of A-FABP and Dio2 in the BAT of WT mice, whereas this induction was obviously impaired in A-FABP KO mice (Fig. 6a–d). On the contrary, LXRα expression decreased substantially in WT mice in response to HFD or cold exposure, but its expression in A-FABP KO mice was not altered (Fig. 6a–d). Furthermore, treatment with rA-FABP did not alter the gene expression of A-FABP while it significantly suppressed LXRα but increased Dio2 expression in both primary A-FABP-deficient and WT brown adipocytes (Fig. 6e). Treatment with the mutant R126Q also showed similar effects on the gene expression in both A-FABP deficient- and WT brown adipocytes (Supplementary Fig. 11), but to a lesser extent compared with those treated with rA-FABP. To assess whether A-FABP represses LXRα activity, WT- or A-FABP deficient primary brown adipocytes were treated with the LXRα agonist TO901317 in the presence or absence of rA-FABP followed by monitoring the expression of the downstream target genes of LXRα, including stearoyl-CoA desaturase (SCD-1) and sterol regulatory element-binding transcription factor 1 (SREBP-1c)34,35. Treatment with TO901317

Figure 5 | A-FABP deficiency impairs conversion of T4 to T3 in BAT of mice. (a) Circulating T4 and (b) T3 levels of male 4-week-old A-FABP KO mice and WT littermates fed with STC or HFD for 24 weeks as indicated in Fig. 2 (n = 8). (c,d) T3 levels in BAT of male 4-week-old A-FABP KO mice and WT littermates fed with STC or HFD 24 weeks or (d) subjected to cold exposure (6 °C) for 24 h as indicated in Fig. 2 (n = 8). Male 4-week-old A-FABP KO and WT mice fed with HFD for 4 weeks were supplemented with PBS, T4 (400 μg kg−1, 5 days) or T3 (500 μg kg−1, 1 day) followed by cold exposure (6 °C) for 24 h. (e,f) Energy expenditure of mice supplemented with (e) T4 or (f) T3 followed by cold exposure (6 °C) for 24 h (n = 6). (g) Representative IHC staining and densitometry analysis (right panel) for UCP-1 in the BAT of mice. Scale bar, 20 μM, with magnification of 400 ×. Representative images from three independent experiments are shown (n = 6). (h) The mRNA abundance of UCP-1 in BAT of above mice (n = 6). (i,j) T3 levels in BAT isolated from above WT and A-FABP KO mice fed with HFD for 4 weeks supplemented with (i) T3 or (j) T4 followed by cold exposure (6 °C) for 24 h (n = 6). Data are represented as mean ± s.e.m. *P < 0.05, **P < 0.01 (one-way analysis of variance with Bonferroni correction for multiple comparisons).
drastically increased the expression of *SCD-1* and *SREBP-1c* in primary brown adipocytes derived from both genotypes, which were greatly suppressed by co-treatment with rA-FABP (Fig. 6f). Consistently, the downregulated expression of *Dio2* by treatment with TO901317 was significantly reversed by rA-FABP (Fig. 6f), suggesting that A-FABP increases *Dio2* expression via inhibition of *LXR*.

A-FABP promotes proteasomal degradation of *LXR*.

To further explore the potential mechanism whereby A-FABP inhibits *LXR*, A-FABP deficient- and WT primary adipocytes were treated with the transcription inhibitor actinomycin D followed by measuring the mRNA abundance of *LXR* at different time points. A-FABP deficiency did not have significant effect on the basal mRNA level of *LXR*. Furthermore, the mRNA abundance of *LXR* was gradually decreased to a similar extent in both WT and A-FABP deficient adipocytes after treatment with actinomycin D (Fig. 7a), suggesting that A-FABP does not alter the mRNA stability of *LXR*. We then examined if A-FABP modulates the protein stability of *LXR* using cycloheximide (a protein synthesis inhibitor) chase assay. The degradation of *LXR* in A-FABP deficient primary brown adipocytes after treatment with cycloheximide was significantly attenuated compared to that of the WT adipocytes (Fig. 7b), suggesting that the presence of A-FABP accelerates protein degradation of *LXR*.

**Figure 6** | A-FABP mediates expression of *Dio2* via inhibition of *LXR*. (a,b) BAT isolated from WT and A-FABP KO mice (a) fed with STC or HFD for 24 weeks or (b) subjected to room temperature (23 °C) or cold exposure (6 °C) for 24 h as indicated in Fig. 2 were subjected to immunoblotting using an antibody against A-FABP, type II iodothyronine deiodinase (D2), liver X receptor α (*LXRα*) and β-tubulin. The bar charts below are the band intensity of each protein relative to β-tubulin and expressed as arbitrary units, N.D., not detected (*n* = 6). (c,d) The mRNA abundance of A-FABP, *Dio2* and *LXRα* in BAT of above WT and A-FABP KO mice (c) fed with STC or HFD for 24 weeks or (d) subjected to cold exposure (6 °C) for 24 h (*n* = 6). (e) The mRNA abundance of A-FABP, *LXRα* and *Dio2* in WT or A-FABP-deficient primary brown adipocytes incubated with PBS or recombinant A-FABP (rA-FABP, 2 μg ml⁻¹) for 24 h (*n* = 6). (f) The mRNA abundance of *LXR* downstream target genes (*SCD-1*, *SREBP-1c*) and *Dio2* in WT and A-FABP-deficient primary brown adipocytes treated with or without *LXR* agonist TO901317 (TO;1 μM) and/or rA-FABP (2 μg ml⁻¹) for 24 h (*n* = 6). Uncropped western blot images are shown in Supplementary Fig. 13. Data are represented as mean ± s.e.m. *P* < 0.05, **P** < 0.01 (One-way analysis of variance with Bonferroni correction for multiple comparisons).
LXRα. Conversely, treatment of cycloheximide together with MG132 (an inhibitor that reduces proteasomal degradation of ubiquitin-conjugated proteins) blocked the degradation of LXRα in both WT and A-FABP-deficient primary adipocytes (Fig. 7c). Furthermore, adenovirus-mediated overexpression of A-FABP significantly enhanced the degradation of LXRα in A-FABP-deficient primary adipocytes comparing with its controls with overexpression of luciferase (Ad-Luci) for 48 h, followed by treatment with CHX (50 μg/ml) for 0, 6, 12 and 24 h, and then subjected to immunoblotting using an antibody against LXRα, A-FABP and GADPH as indicated (n = 4). The right panel is the band intensity of LXRα normalized with GAPDH, and expressed as percentage relative to baseline (0 h). Uncropped western blot images are shown in Supplementary Fig. 14. Data are represented as mean ± s.e.m. *P < 0.05, **P < 0.01 (Students’ t-test).

**A-FABP restores T4-induced energy expenditure in KO mice.**

To confirm the role of A-FABP in intracellular conversion of T4 to T3 in BAT, HFD-fed A-FABP KO and WT mice were infused with either rA-FABP or PBS (as vehicle), followed by subcutaneous injection of T4 for 5 consecutive days and cold exposure for another 24 h (Fig. 8a). Energy expenditure increased significantly in T4-treated WT mice comparing with vehicle-treated controls (Fig. 8b,c). Consistent with the above result (Fig. 5e), the oxygen consumption of T4-treated A-FABP KO mice was comparable to that of WT mice without T4 treatment, while replenishment of rA-FABP together with T4 significantly increased oxygen consumption of A-FABP KO mice similar to that in T4-treated WT mice (Fig. 8b,c), suggesting that A-FABP is essential for the effect of T4 on induction of energy expenditure in vivo. Moreover, T4-induced increase in multi-locular cells and expression of UCP-1 in BAT was significantly augmented by infusion of rA-FABP in A-FABP KO mice (Fig. 8d).

---

**Figure 7 | A-FABP accelerates proteasomal degradation of LXRα.** (a) Primary adipocytes derived from male 6-week-old A-FABP KO mice or WT littermates were treated with actinomycin D (actD, 1 μg ml⁻¹) or vehicle (PBS). The mRNA level of LXRα was determined by real-time PCR at time points as indicated (n = 4). (b, c) Primary brown adipocytes derived from male 6-week-old A-FABP KO mice or WT littermates were treated with (b) cycloheximide (CHX, 50 μg ml⁻¹) or (c) CHX (50 μg ml⁻¹) together with MG132 (10 μM) for different periods were subjected to immunoblotting using an antibody against LXRα, A-FABP and GADPH as indicated (n = 4). (d) Primary brown adipocytes derived male 6-week-old A-FABP KO mice or WT littermates were infected with adenovirus overexpressing A-FABP (Ad-AFABP) or luciferase (Ad-Luci) for 48 h, followed by treatment with CHX (50 μg ml⁻¹) for 0, 6, 12 and 24 h, and then subjected to immunoblotting using an antibody against LXRα, A-FABP and GADPH as indicated (n = 4). The right panel is the band intensity of LXRα normalized with GAPDH, and expressed as percentage relative to baseline (0 h). Uncropped western blot images are shown in Supplementary Fig. 14. Data are represented as mean ± s.e.m. *P < 0.05, **P < 0.01 (Students’ t-test).
In line with our in vitro findings (Figs 6 and 7), replenishment with rA-FABP significantly attenuated the expression of LXRα in A-FABP KO mice, which was accompanied by enhanced T4-induced expression of Dio2 and UCP-1 in BAT (Fig. 8e). Replenishment with rA-FABP did not alter the circulating levels of T4 or T3 in A-FABP KO mice (Fig. 8f,g). However, with the pre-treatment of T4, the intracellular T3 level in BAT of A-FABP KO mice infused with rA-FABP was significantly elevated compared with A-FABP KO mice infused with PBS (Fig. 8h), indicating that A-FABP controls the intracellular conversion of T4 to T3 in BAT. Similar results were also observed in T4-treated WT and A-FABP KO mice with or without replenishment with rA-FABP.
In transport of FFAs is largely ignored, despite the fact that diet-induced body weight gain in mice. Deletion of A-FABP is also shown to impair thermogenesis in mice under fasting state28. Although the abundant expression of both LXRα and LXRβ receptors is a well-established target of thyroid hormone42. The preponderance of thyroid hormones released from the thyroid glands into bloodstream is its inactive form T4, which needs to be converted intracellularly by D2 into its bioactive metabolite T3 for further activation of thyroid hormone receptors43. In BAT, thyroid hormones act coordinately with SNS to promote thermogenesis. Upon thermogenic stimuli, activation of SNS promotes the stimulatory effects of catecholamines on thermogenesis in BAT6. Activated thyroid hormone receptors also act directly to increase cAMP-mediated induction of the expression of UCP-1 gene and to induce the expression of a cluster of genes involved in mitochondrial biogenesis37,46. Our present study found that thermogenic stimuli (such as cold and HFD challenge)-induced expression of D2 and conversion of T4 to T3 in BAT were markedly decreased in A-FABP KO mice. Furthermore, supplementation of T4 significantly stimulated cold-induced oxygen consumption and BAT activation in WT mice, but not in A-FABP KO mice, although A-FABP KO and WT mice were equally sensitive to norepinephrine-induced BAT activation and thermogenesis. In support of our conclusion, phenotypic changes of D2 KO mice are also strikingly similar to A-FABP KO mice in our study, including diminished conversion of T4 to T3 in BAT, and impaired adaptive thermogenesis in response to cold challenge despite normal serum T3 levels7.

Our study demonstrated that the effects of A-FABP on promoting D2 expression and T4 to T3 conversion were mediated

Figure 9 | Mechanisms by which A-FABP regulates adaptive thermogenesis. In response to cold challenge or HFD, A-FABP is elevated in BAT, WAT and in the circulation. Elevated A-FABP in BAT induces the expression of Dio2 via suppression of LXRα. Increased Dio2 promotes adaptive thermogenesis by enhancing conversion of T4 to T3 in BAT.

In addition, elevated circulating A-FABP facilitates the delivery of WAT-derived FFAs to BAT. Increased FFAs supply to BAT further induces the expression and activation of UCP-1 for thermogenesis.
by its suppression of LXRα, which is a nuclear receptor having a key role in regulating bile acid, glucose and lipid homeostasis. In BAT of A-FABP KO mice, the impairment in cold-induced expression of D2 was accompanied by an attenuated reduction of LXRα. Vice versa, the effect of LXRα agonist TO901317 on suppression of Dio2 in primary brown adipocytes was abrogated by treatment with rA-FABP. Activation of LXRα suppresses the transcription of the Dio2 gene by binding to its promoter, thus reducing D2-mediated T3 production in BAT. In line with our study, LXR α/β –/– mice display increased energy expenditure and UCP-1 expression in BAT while treatment with the LXR agonist GW3965 exerted opposite effects. The expression of Dio2 is also sixfold higher in the LXRα –/– mice compared with their WT littermates. Furthermore, LXRα forms complex with its co-factor, the receptor interacting protein 140, which competes with peroxisome proliferator-activated receptor γ (PPARγ) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) for the enhancer region of the UCP-1 gene promoter and represses the gene expression. Therefore, A-FABP may also enhance UCP-1 expression through its suppressive effect on LXRα.

Consistent with our findings, A-FABP has been shown to downregulate LXRα activity in macrophages, leading to altered de novo lipogenesis and ER stress and exacerbated atherosclerosis. Furthermore, A-FABP mediates ubiquitination and the subsequent proteasomal degradation of PPARγ and LXRα can be ubiquitinated by BRCA1-associated Ring domain/brassinosteroid receptor-1 (BRCA1-associated Ring domain/brassinosteroid receptor-1) and its stability and activity can be regulated by ubiquitination-mediated proteasomal degradation suggesting that A-FABP promotes ubiquitination-dependent proteasomal degradation of LXRα as one of the mechanisms by which A-FABP suppresses LXRα. Further studies are needed to investigate how A-FABP accelerates the proteosomal degradation of LXRα by modulating ubiquitination. Notably, A-FABP is also a downstream target of LXRα activation, suggesting the existence of a negative feedback loop between A-FABP and LXRα. It is also worthy to note that unsaturated FFAs can act as the suppressor of LXRα by preventing its binding to the target genes. As we showed that A-FABP can form complex with circulating FFAs and facilitates their uptake into BAT, it is also possible that A-FABP-mediated accumulation of intracellular unsaturated FFAs interferes with the binding of LXRα to its responsive element on Dio2 promoter, thereby increasing Dio2 expression in BAT. In addition, A-FABP can modulate the activity of several transcription factors, including jun kinase 2 and PPARγ, the latter of which is also a regulator of LXRα expression. Further investigations are warranted to explore the involvement of these transcription factors in mediating A-FABP-mediated suppression of LXRα and subsequent induction of the Dio2 gene in BAT.

While our present study showed the salutary effects of A-FABP on prevention of obesity through promotion of adaptive thermogenesis in BAT, A-FABP actions in other tissues have been shown to exacerbate obesity-related cardiometabolic disorder via its pro-inflammatory activities. In macrophages, A-FABP potentiates toxic lipids- and endotoxin-induced activation of inflammatory pathways (NF-κB and c-Jun N-terminal kinase) and production of pro-inflammatory cytokines. Ablation of A-FABP in macrophage alone is sufficient to render apolipoprotein E deficient mice refractory to spontaneous development of atherosclerosis. The secreted form of A-FABP can also act on endothelium cells to induce endothelial dysfunction, on cardiomyocytes to suppress cardiac contraction and to mediate cardiac dysfunction during ischemic injury and on hepatocytes to promote gluconeogenesis leading to altered glucose homeostasis. On the other hand, circulating A-FABP may possess insulinotropic action mediating glucose-stimulated insulin secretion of pancreatic β cells. Therefore, these findings, together with our present study, highlight the complex functions of A-FABP in obesity and its associated cardiometabolic disorders due to its differential effects on various target tissues at different stages of the disease. It is likely that elevated A-FABP under physiological stimuli (such as cold challenge) or early phase of obesity may serve as a defense response to promote adaptive thermogenesis through its actions in adipocytes. However, with the progression of obesity, prolonged and excessive increase of A-FABP may exacerbate metabolic and cardiovascular disorders through its effects on non-adipose tissues, including macrophages, endothelium, cardiomyocytes and hepatocytes. The dual effects of A-FABP on obesity and its associated medical complications are strikingly reminiscent of leptin, another adipocyte-secreted hormone that is elevated in both animals and human with obesity.

In conclusion, our study demonstrated an important role of adipocyte-derived A-FABP in adaptive thermogenesis, via its actions on conversion of T4 to T3 by modulating the LXRα-Dio2 signalling axis and facilitating the uptake of circulating FFAs into BAT. Notably, the pharmacological inhibitors of A-FABP such as BMS390403 (ref. 67) and the recent identified A-FABP monoclonal antibody CA33 (ref. 68) alleviate metabolic and cardiovascular disorders in animals. Our study suggests that global pharmacological inhibition of A-FABP may not be an optimal therapeutic strategy for obesity-related cardiovascular and metabolic diseases due to the potential impairment of adaptive thermogenesis. Further investigations to dissect the structural and molecular basis underlying the differential effects of A-FABP in different tissues are needed in order to design more effective therapeutic interventions for obesity and its related medical complication by targeting A-FABP.

Methods

Animals. A-FABP KO mice in C57BL/6J background were generated using the same procedures as previously described. Age-matched male A-FABP KO mice and their littermates were used in all the experiments of this study. Animals were allocated to their experimental group according to their genotypes. No randomization of mice was used. The investigators were not blinded to the experimental groups. Mice were housed in a temperature-controlled facility (23 °C, 12-hour light/dark cycle, 60–70% humidity). Four-week-old mice were weaned and fed with either STC (Purina, Framingham, MA, USA) or Western diet (D12079B, Research Diet, USA) for 4 or 24 weeks. Body composition was determined bi-weekly by nuclear magnetic resonance (Bruker,.minispec, Germany). All experimental protocols were approved by the Committee on the Use of Live Animals in Teaching and Research at the University of Hong Kong.

Cold exposure. Male 4-week-old A-FABP KO mice and their littermates fed with STC or HFD for 24 weeks were provided with food and water ad libitum at 6 °C for 8 or 24 h. Rectal temperature was measured with 4610 Precision Thermometer (Thermo Scientific, MA, USA), and serum was collected at various time points via tail vein for lipid profile analysis.

Glucose tolerance test and insulin tolerance test. For glucose tolerance test, male 4-week-old A-FABP KO mice and their littermates fed with STC or HFD for 24 weeks were housed in clean cages with fasting for 16 h before intra-peritoneally injected with D-glucose (1 g kg⁻¹). Blood glucose was monitored at 0, 10, 20, 30, 45, 60, 75 and 90 min after glucose injection. Male 4-week-old A-FABP KO mice and their littermates fed with HFD for 4 weeks infused with rA-FABP (196 g liter⁻¹) or its mutant R126Q (196 g liter⁻¹) were also subjected to glucose tolerance test. For insulin tolerance test, male 4-week-old A-FABP KO mice and their littermates fed with STC or HFD for 24 weeks were fasted
for 6 h followed by intra-peritoneal injection of insulin (1 U kg⁻¹ for mice fed with HFD and 0.5 U kg⁻¹ for mice fed with STC). Blood glucose was measured at 0, 20, 40, 60 and 80 min after insulin injection. HOMA index was calculated according to the formula: fasting insulin (micro U L⁻¹) × fasting glucose (mM L⁻¹)/22.5.

**Indirect calorimetry.** Whole-body oxygen consumption (VO₂) was assessed using Indirect Calorimetry with the Columbus Comprehensive Lab Animal Monitoring System (CLAMS, Columbus, USA) as previously described²,³. Briefly, male 4-week-old A-FABP KO mice and their littermates fed with STC or HFD for 4 weeks, mice were acclimated to CLAMS cages individually with food and water ad libitum for 24 h. Data on OCR (VO₂) were recorded every 10 or 11 min for a 48-hour period at 23 °C or 6 °C. Physical activity was measured by infrared technology (OPT-M3, Columbus Instruments). Norepinephrine-induced energy expenditure was determined. Briefly, male 4-week-old A-FABP KO mice and their littermates fed with STC or HFD for 24 weeks were anaesthetized and VO₂ was recorded for the first 30 min to access basal energy expenditure. Individual mice were then injected with norepinephrine (1 mg kg⁻¹, Sigma-Aldrich), and VO₂ was determined for another 60 min.

**Generation of rA-FABP and A-FABP mutant R126Q.** Mice A-FABP (GenBank BC054426.1) was cloned into the His-tag expression vector pDEST17. A-FABP mutant R126Q was generated using Quick Change Multi Site-Directed Mutagenesis kit (Stratagene, La Jolla, California, USA) for 30 min at 37 °C. The SVFs were cultured in Dulbecco’s Modified Eagle Medium (DMEM) with 10% fetal bovine serum (125 nM, Sigma-Aldrich), imidazole (Sigma-Aldrich), indomethacin (125 nM, Sigma-Aldrich), retinoic acid (20 μM, Sigma-Aldrich) and Vitamin C (142 μM, Sigma-Aldrich) in DMEM for 2 days then changed to DMEM containing insulin (20 μM) and T3 (1 nM) for another 4 days.

**Replenishment of rA-FABP and thyroid hormones.** Thyroxine (T₄, 400 μg kg⁻¹, 5 days, Sigma-Aldrich) or T₃ (500 μg kg⁻¹, 1 day, Sigma-Aldrich) were administered into male 4-week-old A-FABP KO mice and their WT littermates fed with HFD for 4 weeks by subcutaneous injection. The rA-FABP or A-FABP mice were infected with 0.5 U kg⁻¹ rA-FABP (2 μg ml⁻¹) or palmitate (200 μM, Sigma-Aldrich) for 30 min. Oligomycin (5 μM, ATP synthase inhibitor, Sigma-Aldrich), carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP, 50 μM, cellular uncoupler, Sigma-Aldrich), rotenone/antimycin A (1 μM, Sigma-Aldrich) were sequentially added to determine basal- ATP-dependent, maximal- and mitochondria-independent oxygen consumption, respectively.

**Palmitate uptake in mice and primary adipocytes.** Male 8-week-old A-FABP KO mice and their relative WT littermates infected with rA-FABP (1 μg g⁻¹ or R126Q (1 μg g⁻¹) or PBS) for 14 days were orally administrated with 200 μl olive oil containing Palmitate (2 μg, PerkinElmer, USA) for 4 h in the absence of food and water. Interscapular BAT, subcutaneous WAT and epididymal WAT and various peripheral tissues were freshly isolated and minced for measurement of radioactivity. For in vivo FA tracing, BODIPY-FA (20 μM) was pre-incubated with or without rA-FABP (50 μg) or A-FABP mutant R126Q (30 μg) for 30 min. These BODIPY-FA were injected through tail vein into male 8-week-old A-FABP KO mice, and the fluorescence was measured by the PE IVIS Spectrum in vivo imaging system (PerkinElmer, USA). For palmitate uptake in primary adipocytes, 3H-palmitate (55 mCi mmol⁻¹, PerkinElmer, USA) or BODIPY-FA (2 μM, Molecular Probes) was pre-incubated with BSA (3 μg ml⁻¹ or rA-FABP (2 μg ml⁻¹) 30 min before adding to differentiated primary adipocytes for 10 min. Radioactivity or fluorescence of cell lysates was measured by Liquid Scintillation Counter (PerkinElmer, USA) or Infinite M200 Microplate Reader (Tecan Systems, Inc. San Jose, CA, USA), respectively. For tracing of BODIPY-FA and A-FABP, rA-FABP was fluorescent-labelled using Alexa Fluor 488 Protein Labelling Kit (Invitrogen, CA, USA), BODIPY-FA (2 μM) was washed with or without fluorescent-labelled rA-FABP (2 μg ml⁻¹) for 30 min, and then was added to rA-FABP-deficient primary brown adipocytes. The fluorescent images of cells were obtained at different time points using a microscope (Bx41 System, Olympus) with a colour digital camera (Olympus Model DP72).

**14C-glucose uptake.** Male 4-week-old A-FABP KO mice and their WT littermates fed with HFD for 4 weeks were intra-peritoneally injected with 2-[1-14C] deoxy-D-glucose (20 μCi, PerkinElmer, USA) for 2 h in the absence of food and water. Interscapular BAT, liver and soleus muscle were isolated and minced for measurement of radioactivity and normalized with protein concentrations.

**Immuno blot analysis and real-time PCR.** Proteins were separated by SDS–polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes, and probed with primary antibodies A-FABP (0.25 μg ml⁻¹, goat polyclonal, AbD Serotec, USA), LXRα (0.25 μg ml⁻¹, rabbit monoclonal, Abcam, Cambridge, UK), UCP-1 (0.5 μg ml⁻¹, rabbit polyclonal, ab77481, Abcam), type II iodothyronine deiodinase (D2, 0.25 μg ml⁻¹, rabbit polyclonal, ab77481, Abcam), tyrosine transaminase (0.25 μg ml⁻¹, rabbit polyclonal, ab21355, Abcam), tyrosine hydroxylase (0.25 μg ml⁻¹, rabbit polyclonal, ab51204, Abcam), short chain fatty acid-CoA ligase (0.25 μg ml⁻¹, rabbit polyclonal, ab17153, Abcam), T2, T3 (500 μg ml⁻¹, rabbit monoclonal, 5174, Cell Signaling), 14-3-3σ (1 μg ml⁻¹, rabbit monoclonal, ab32051, Cell Signaling), 14-3-3τ (1 μg ml⁻¹, rabbit monoclonal, ab32051, Cell Signaling). The intensities of protein bands were quantified using the NIH Image J software.

Total RNA was extracted with Trizol (Invitrogen) and reverse transcribed into complementary DNA using ImProm II reverse transcription kit (Promega, Madison, USA). Real-time PCR was performed using SYBR Green master mix (Qagen, Venlo, the Netherlands) on a 7,900 HT (Applied Biosystems, CA, USA), normalized against the G3PDH gene. Primer sequences are listed in Supplementary Table 1.

**Analysis of mRNA stability in primary brown adipocytes.** SVFs isolated from male 6-week-old A-FABP KO mice and their relative WT littermates differentiated into primary brown adipocytes in 12-well plates. Differentiated adipocytes were treated with actinomycin D (2 μg ml⁻¹) or vehicle (PBS). The mRNA abundance of LXRα was measured at various time points (0, 4, 6, 12 h) by real-time PCR.
Analysis of protein stability in primary brown adipocytes. SVFs isolated from male 6-week-old A-FABP KO mice and their WT littermates were differentiated into primary brown adipocytes in 12-well plates. WT and A-FABP-deficient primary adipocytes were treated with cycloheximide (50 μg/ml −1, Sigma-Aldrich) in the presence or absence of the proteasome inhibitor MG132 (10 μM, Sigma-Aldrich). In addition, A-FABP-deficient primary adipocytes were infected with adenovirus over-expressing A-FABP (Ad-AFABP) or lucfascer (Ad-Luci) at fifty multiplicity of infection (M.O.I) for 48 h followed by treatment with cycloheximide (50 μg/ml −1). Adipocytes were harvested at indicated time points and the expression of LXRα and A-FABP was determined by western blot analysis.

Histological and immunohistochemistry analysis. Paraffin-embedded adipose tissues were prepared at the thickness of 5 μm. Deparaffinized and dehydrated sections were stained with haematoxylin and eosin (Sigma-Aldrich) as previously described. 18 For immunocytochemistry, sections were sequentially incubated with primary antibody UCP-1 (1 μg/ml −1, rabbit polyclonal; Abcam, UK) overnight and anti-rabbit secondary antibody (4 μg/ml −1; Cell Signaling Technology) for 1 h at 23 °C, followed by development with 3, 3’ diamobenzidine solution (Sigma-Aldrich). The nuclei were counter-stained with haematoxylin. The intensities of positively stained cells were quantified in each of five randomly selected fields by the Image J software. Two independent investigators blinded to sample identity, one investigator performed the staining and another investigator analysed the adipose tissue sections.

Biochemical and immunological analysis. Serum insulin, adiponectin and A-FABP levels were measured using Advanced Ultra Sensitive Mouse Insulin Immunoassay kit, mouse adiponectin ELISA kit (AIS, HKU; Hong Kong) and mouse A-FABP ELISA kit (BioVendor Laboratory Medicine, Modrice, Czech Republic) respectively. T4 and T3 levels in serum or adipose tissues were analysed using mouse T4 or T3 ELISA kit, respectively (Calbiotech, Spring Valley, CA, USA). Plasma glucose was measured using an ACCU-Check glucose meter (Roche, Indianapolis, IN, USA). Serum FFAs, triglyceride and cholesterol were determined using mouse A-FABP ELISA kit (Roche, USA), Stabio Lipocolour Triglyceride and Stabio Lipicolour Cholesterol (STANBIO Laboratory, USA), respectively.

Statistical analysis. All statistical analyses were performed using Prism 6 (GraphPad Software Inc. La Jolla, CA92037 USA). Data were expressed as mean ± s.e.m. Animal sample size for each study was chosen on the basis of literature documentation of similar well-characterized experiments, and no statistical method was used to predetermine sample size. Statistical significance was assessed by Student’s t-test or one-way analysis of variance with Bonferroni correction for multiple comparisons. A value of P < 0.05 was considered statistically significant. Statistical outlier analysis was calculated using the GraphPad Outlier calculator (http://graphpad.com/quickcalc/Grubb1s.cfm). Those significant outliers were excluded from data analysis.

Data availability. The data supporting the findings of this study are available within the article and its Supplementary Information Files, or are available from the corresponding author upon reasonable request.

References
1. Dietrich, M. O. & Horvath, T. L. Limitations in anti-obesity drug development: the critical role of hunger-promoting promoters. Nat. Rev. Drug Discov. 11, 673–691 (2012).
2. Nedergaard, J. et al. UCP1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic inefficiency. Biochim. Biophys. Acta 1504, 82–106 (2001).
3. Lowell, B. B. & Spiegelman, B. M. Towards a molecular understanding of adaptive thermogenesis. Nature 404, 652–660 (2000).
4. Wijers, S. L., Saris, W. H. & van Marken Lichtenbelt, W. D. Recent advances in within the article and its Supplementary Information Files, or are available from
ARTICLE

38. Syamsumarno, M. R. et al. A critical role of fatty acid binding protein 4 and 5 (FABP4/5) in the systemic response to fasting. PLoS ONE 8, e79386 (2013).

39. Richieri, C. V., Ogata, R. T. & Kleinfeld, A. M. Kinetics of fatty acid interactions with fatty acid binding proteins from adipocyte, heart, and intestine. J. Biol. Chem. 271, 11291–11300 (1996).

40. Putri, M. et al. CD36 is indispensable for thermogenesis under conditions of fasting and cold stress. Biochem. Biophys. Res. Commun. 457, 520–525 (2015).

41. Wu, Q. et al. Fatty acid transport protein 1 is required for nonshivering thermogenesis in brown adipose tissue. Diabetes 55, 3229–3237 (2006).

42. Bianco, A. C. & Silva, J. E. Nuclear 3, 5′-triodothyronine (T3) in brown adipose tissue: receptor occupancy and sources of T3 as determined by in vivo techniques. Endocrinology 120, 55–62 (1987).

43. Bianco, A. C. & Silva, J. E. Intracellular conversion of thyroxine to triiodothyronine is required for the optimal thermogenic function of brown adipose tissue. J. Clin. Invest. 79, 295 (1987).

44. Silva, J. & Larsen, P. Adrenergic activation of triiodothyronine production in brown adipose tissue. Nature 305, 712–713 (1983).

45. Rubio, A., Raasmaja, A. & Silva, J. E. Thyroid hormone and norepinephrine signaling in brown adipose tissue. Dev. Biol. 136, 3277–3284 (1990).

46. Lopez, M., Alvarez, C. V., Nogueiras, R. & Dieuguez, C. Energy balance regulation by thyroid hormones at central level. Trends Mol. Med. 19, 418–427 (2013).

47. Laurenti-Giusti, M. S. The fatty acid-binding protein, aP2, coordinates macrophage cholesterol efflux and atherogenesis. Mol. Cell Biochem. 236, 137–146 (2002).

48. Andre, M., Archer, A., Barros, R. P., Parini, P. & Gustafsson, J. A. Both liver X receptors and fat cell metabolism. J. Obes. (Lond) 326–334 (2009).

49. Wang, H. Liver X receptor ligands suppress ubiquitination and degradation of LXRalpha by displacing BARD1/BRCA1. Mol. Cell Biochem. 1, 3229–3237 (2006).

50. Garin-Shkolnik, T., Rudich, A., Hotamisligil, G. S. & Rubinstein, M. FABP4 degradation of LXRalpha by displacing BARD1/BRCA1. Mol. Cell Biochem. 1 cells.

51. Kim, H. K. et al. Liver X receptor ligands suppress ubiquitination and degradation of LXRalpha by displacing BARD1/BRCA1. Mol. Endocrinol. 23, 467–479 (2009).

52. Liu, Q.-Y., Quinet, E. & Nambi, P. Adipocyte fatty acid-binding protein (aP2), a newly identified LXR target gene, is induced by LXR agonists in human THP-1 cells. Mol. Cell Biochem. 302, 203–213 (2007).

53. Ou, J. et al. Unsurpassed fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c (SREBP-1c) gene by antagonizing ligand-dependent activation of the LXR. Proc. Natl Acad. Sci. USA 98, 6027–6032 (2001).

54. Thompson, B. R., Mazurkiewicz-Munoz, A. M., Suttles, J., Carter-Su, C. & Bernlohr, D. A. Interaction of adipocyte fatty acid-binding protein (AFABP) and JAK2: AFABP/aP2 as a regulator of JAK2 signaling. J. Biol. Chem. 284, 13473–13480 (2009).

55. Garin-Shkolnik, T., Rudich, A., Hotamisligil, G. S. & Rubinstein, M. FABP4 attenuates PPARgamma and adipogenesis and is inversely correlated with PPARgamma in adipose tissues.

56. Chawla, A. et al. A PPARγ-LXR-ABCa1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol. Cell 7, 161–171 (2001).

57. Makowski, L., Brittingham, K. C., Reynolds, J. M., Wu, Q. & Tabas, I. Fatty acid binding protein 2, coordinates macrophage cholesterol trafficking and inflammatory activity. Macrophage expression of aP2 impacts perilipase proliferator-activated receptor gamma and ikappab kinase activities. J. Biol. Chem. 280, 12888–12895 (2005).

58. Makowski, L. et al. Lack of macrophage fatty acid-binding protein 2 protects mice deficient in apolipoprotein E against atherosclerosis. Nat. Med. 7, 699–705 (2001).

59. Lee, M. Y. et al. Chronic administration of BMS309403 improves endothelial function in apolipoprotein E-deficient mice and in cultured human endothelial cells. Br. J. Pharmacol. 162, 1564–1576 (2011).

60. Lamounier-Zepter, V. et al. Adipocyte fatty acid-binding protein suppresses cardiomyocyte contraction: a new link between obesity and heart disease. Circ. Res. 105, 326–334 (2009).

61. Wu, L. E. et al. Identification of fatty acid binding protein 4 as an adipokine that regulates insulin secretion during obesity. Mol. Metab. 3, 465–473 (2014).

62. Friedman, J. M. & Halaas, J. L. Leptin and the regulation of body weight in mammals. Nature 395, 763–770 (1998).

63. Sinha, M. K. et al. Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects. J. Clin. Invest. 97, 1344 (1996).

64. Cettoz-Rose, P., Burger, A. G., Meier, C. A., Visser, T. J. & Rohner-Jeanrenaud, F. Central stimulatory effect of leptin on T3production is mediated by brown adipose tissue type 2 deiodinase. Am. J. Physiol. Endocrinol. Metab. 283, E980–E987 (2002).

65. Kaplan, L. M. Leptin, obesity, and liver disease. Gastroenterology 115, 997–1001 (1998).

66. de Gusmao Correia, M. L. & Haynes, W. G. Leptin, obesity and cardiovascular disease. Curr. Opin. Nephrol. Hypertens. 12, 215–223 (2004).

67. Burak, M. F. & Bernlohr, D. A. Interaction of adipocyte fatty acid-binding protein (AFABP) and JAK2: AFABP/aP2 as a regulator of JAK2 signaling. J. Biol. Chem. 284, 13473–13480 (2009).

68. Laurencikiene, J. & Ryden, M. Liver X receptors and fat cell metabolism. J. Obes. (Lond) 36, 1494–1502 (2012).

69. Wang, H. et al. Liver X receptor alpha is a transcriptional repressor of the uncoupling protein 1 gene and the brown fat phenotype. Mol. Cell Biol. 28, 218290 (2008).

Acknowledgements

This study is supported by the National Key Basic Research Development Program-973 Program (2015CB553603), the French National Research Agency (Research Grants Council (RGC) Joint Research Scheme (A-HKU705/13)), RGC/Collaborative Research Fund (C7055-14G), RGC General Research Fund (HKU171247/14, HKU766812 and HKU767913), Health and Medical Research Fund (02131906), Natural Science Foundation of China (81270862) and Shenzhen Basic Research Grant (JCY20140930112959965).

Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

How to cite this article: Shu, L. et al. A-FABP mediates adaptive thermogenesis by promoting intracellular activation of thyroid hormones in brown adipocytes. Nat. Commun. 8, 14147 doi: 10.1038/ncomms14147 (2017).

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s) 2017

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/