Cyclooxygenase-2 Controls Energy Homeostasis in Mice by de Novo Recruitment of Brown Adipocytes

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Science **328**, 1158 (2010);
DOI: 10.1126/science.1186034

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

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32. We thank Prionics for mAb 6H4, V. Saylor for technical help, A. Aguzzi for mAb POM2, P. Nelson for neuropathological expertise, R. Kryscio and J. Marcum for biostatistical advice, and C. Ryou for reading the manuscript. C.S. is currently Founder and Chief Scientific Officer of Amprion Inc., a privately held company focusing on exploiting PMCA for sensitive detection of prions and early disease diagnosis. These studies were supported by grant 2RO1 NS040334-04 from the National Institute of Neurological Disorders and Stroke, grants N01-AI-25491 and 1P01AI077774-01 from the National Institute of Allergy and Infectious Diseases, and grant V180003 from the U.S. Department of Defense. R.C.A. and S.B. were supported by T32 DA022738 and T32 AI49795 Training Programs from the National Institute on Drug Abuse and the National Institute of Allergy and Infectious Diseases, respectively.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1187107/DC1
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14 January 2010; accepted 1 April 2010
Published online 13 May 2010;
10.1126/science.1187107
Include this information when citing this paper.

Cyclooxygenase-2 Controls Energy Homeostasis in Mice by de Novo Recruitment of Brown Adipocytes

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Obesity results from chronic energy surplus and excess lipid storage in white adipose tissue (WAT). In contrast, brown adipose tissue (BAT) efficiently burns lipids through adaptive thermogenesis. Studying mouse models, we show that cyclooxygenase (COX)–2, a rate-limiting enzyme in prostaglandin (PG) synthesis, is a downstream effector of β-adrenergic signaling in WAT and is required for the induction of BAT in WAT depots. PG shifted the differentiation of defined mesenchymal progenitors toward a brown adipocyte phenotype. Overexpression of COX-2 in WAT induced de novo BAT recruitment in WAT, increased systemic energy expenditure, and protected mice against high-fat diet–induced obesity. Thus, COX-2 appears integral to de novo BAT recruitment, which suggests that the PG pathway regulates systemic energy homeostasis.

Obesity arises from chronic energy surplus and excess lipid storage in white adipose tissue (WAT). In contrast to WAT, brown adipose tissue (BAT) burns lipid to generate heat. Until recently, BAT was thought to function primarily in rodents and in newborn babies as a mechanism that facilitates adaptation to cold. However, recent studies have revealed that adult humans also have functional BAT (*1*–*4*), a finding that has fueled speculation that pharmacologic enhancement of BAT development and activity might be a useful strategy to counteract obesity.

In this study, we have explored whether cyclooxygenase-2 (COX-2), a rate-limiting enzyme in prostaglandin (PG) synthesis, contributes to BAT development in mice. Previous work by others had implicated COX-2 in the control of whole-body energy homeostasis and adipose tissue metabolism; for example, selective inhibition of COX-2 was shown to attenuate weight loss and energy expenditure in cancer patients and tumor-bearing mice (*5*, *6*), and genetically manipulated mice that express only one wild-type allele of COX-2 were shown to exhibit fat accumulation (*7*).

These findings prompted us to screen for differential COX-2 expression in WAT obtained from various mouse models of altered energy homeostasis (*8*). No clear differences in COX-2 mRNA expression were detected in mice with genetic or diet-induced obesity or in cachectic mice. However, an increase by a factor of two in COX-2 mRNA was observed in intra-abdominal WAT after 4 weeks of cold exposure (Fig. 1A). This was accompanied by up-regulation of uncoupling protein 1 (UCP1), the major determinant of mitochondrial thermogenesis (Fig. 1B). In the cold, thermogenic inducible BAT (indBAT) is readily engaged in WAT depots upon sympathetic activation of β-adrenergic receptor signaling by norepinephrine (NE) (*9*, *10*). To recapitulate cold exposure, we treated mice with the β_3-adrenoreceptor agonist CL316243 (CL) (*10*). Acute stimulation with CL resulted in a marked induction of COX-2 but not COX-1 mRNA in intra-abdominal WAT (Fig. 1C) and enhanced the release of the major WAT-derived PG (*11*), PGE_2 and PGI_2, from WAT explants (fig. S2). Notably, COX-2 mRNA levels were also induced upon ex vivo stimulation of mature adipocytes with NE, but only marginally in the stromal-vascular cell fraction (SVF) (fig. S3).

We next investigated the effect of prolonged β_3-adrenergic stimulation in wild-type and COX-2–deficient mice (*12*) and in wild-type mice fed a control diet or a diet containing celecoxib (cx), a selective COX-2 inhibitor (*13*). CL treatment resulted in induction of COX-2 protein expression along with a pronounced BAT-like phenotype in WAT of wild-type mice fed control diet, as judged by the predominance of smaller cells with rich cytoplasmic staining, multilocular lipid droplets, and UCP1 expression (Fig. 1, D to F, and fig. S4). BAT characteristics were substantially diminished in WAT of CL-treated animals on a celecoxib diet

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as well as in COX-2$^{-/-}$ mice (Fig. 1, E and F, and fig. S5). Also, in comparison with vehicle control, CL caused a strong reduction of fat accumulation under control diet, whereas the effect of CL under celecoxib diet and COX-2 deficiency was attenuated (fig. S6). These results suggested that de novo indBAT recruitment and the systemic thermogenic response to β-adrenergic stimulation depend on COX-2 activity. Consistent with this interpretation, CL-mediated induction of genes that are critical for cellular thermogenesis (UCP1, CIDEA, CPT1B, and DIO2) (*9*), for brown adipocyte differentiation (CEBPB), or for the genetic activation of thermogenesis (PGC1A and PPARA) (*9*, *14*) was markedly blunted upon pharmacologic or genetic COX-2 inhibition (Fig. 1G and figs. S7 and S8). In contrast, CL-stimulated thermogenic mRNA expression in interscapular BAT, a constitutive BAT (conBAT) depot in mice (*9*), was not influenced by celecoxib (fig. S9).

To explore whether increased COX-2 activity is also sufficient for indBAT recruitment under conditions of steady-state β-adrenergic stimulation, we studied transgenic mice overexpressing the COX-2 gene under the control of the promoter for the keratin 5 gene (K5COX2) (*15*). In the absence of systemic inflammation (fig. S10), the K5COX2 model mimicked the CL-induced elevation of WAT PG levels (fig. S11) and of COX-2 expression in multilocular adipocytes within intra-

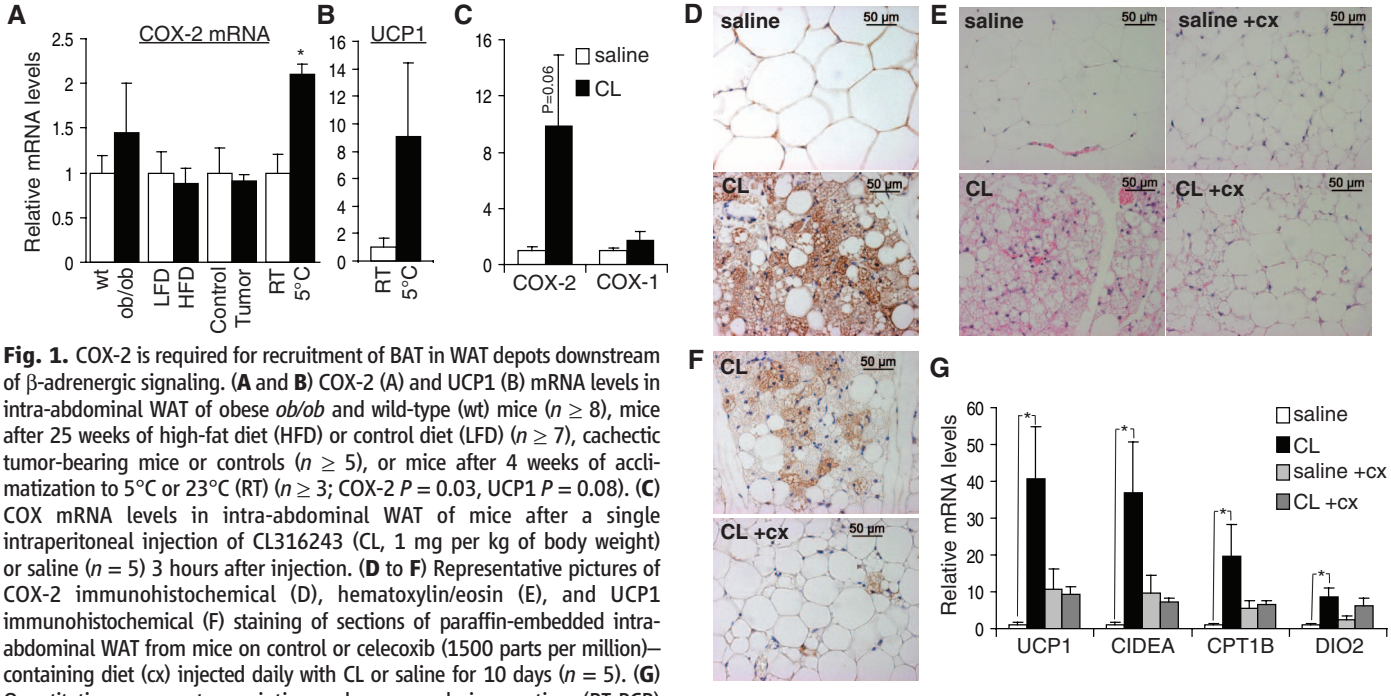

Fig. 1. COX-2 is required for recruitment of BAT in WAT depots downstream of β-adrenergic signaling. (**A** and **B**) COX-2 (A) and UCP1 (B) mRNA levels in intra-abdominal WAT of obese *ob/ob* and wild-type (wt) mice ($n \geq 8$), mice after 25 weeks of high-fat diet (HFD) or control diet (LFD) ($n \geq 7$), cachectic tumor-bearing mice or controls ($n \geq 5$), or mice after 4 weeks of acclimatization to 5°C or 23°C (RT) ($n \geq 3$; COX-2 $P = 0.03$, UCP1 $P = 0.08$). (**C**) COX mRNA levels in intra-abdominal WAT of mice after a single intraperitoneal injection of CL316243 (CL, 1 mg per kg of body weight) or saline ($n = 5$) 3 hours after injection. (**D** to **F**) Representative pictures of COX-2 immunohistochemical (D), hematoxylin/eosin (E), and UCP1 immunohistochemical (F) staining of sections of paraffin-embedded intra-abdominal WAT from mice on control or celecoxib (1500 parts per million)–containing diet (cx) injected daily with CL or saline for 10 days ($n = 5$). (**G**) Quantitative reverse transcription polymerase chain reaction (RT-PCR) mRNA analysis of intra-abdominal WAT from same mice as in (D) to (F) [$n \geq 4$; analysis of variance (ANOVA) post tests, $^*P < 0.05$]. Means ± SEM.

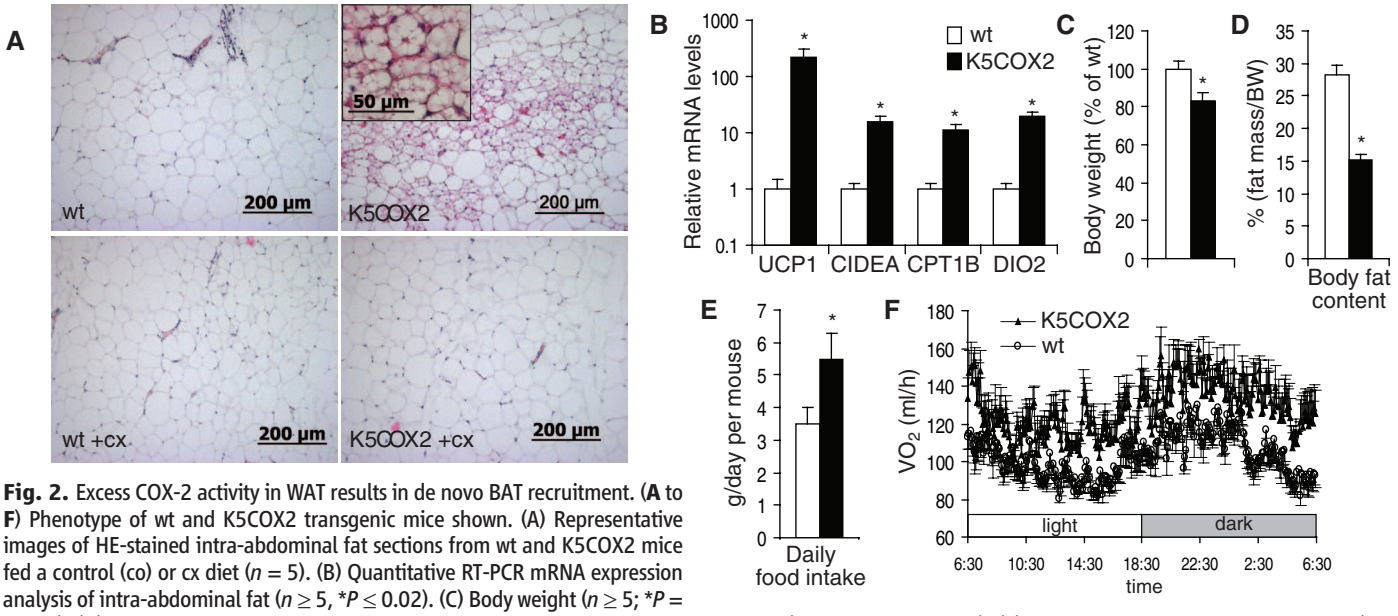

Fig. 2. Excess COX-2 activity in WAT results in de novo BAT recruitment. (**A** to **F**) Phenotype of wt and K5COX2 transgenic mice shown. (A) Representative images of HE-stained intra-abdominal fat sections from wt and K5COX2 mice fed a control (co) or cx diet ($n = 5$). (B) Quantitative RT-PCR mRNA expression analysis of intra-abdominal fat ($n \geq 5$, $^*P \leq 0.02$). (C) Body weight ($n \geq 5$; $^*P = 0.015$). (D) Body fat content determined by nuclear magnetic resonance quantitation ($n = 17$; $^*P < 0.0001$). (E) Daily chow consumption per mouse ($n \geq 6$; $P = 0.04$). (F) Oxygen consumption rate of individual mice determined during a 24-hour period ($n \geq 6$; $P = 0.002$ for wt versus K5COX2 area under the curve). Means ± SEM.

abdominal WAT (fig. S12), which could be attributed to a positive feedback loop of PG on local COX-2 expression (figs. S1 and S11 to S14). Clusters of BAT-like cells were detectable within WAT in intra-abdominal fat sections from K5COX2 mice (Fig. 2A). Correlating with loss of COX-2 expression in WAT and normalized plasma PG levels (figs. S12 and S13), BAT-like cells were absent in intra-abominal fat upon "therapeutic" celecoxib exposure (Fig. 2A). Consistently, mRNA expression analysis showed a marked induction of the thermogenic gene expression program in K5COX2 mice (Fig. 2B and fig. S15), which suggests that local COX-2 overexpression in WAT is sufficient for ectopic indBAT development.

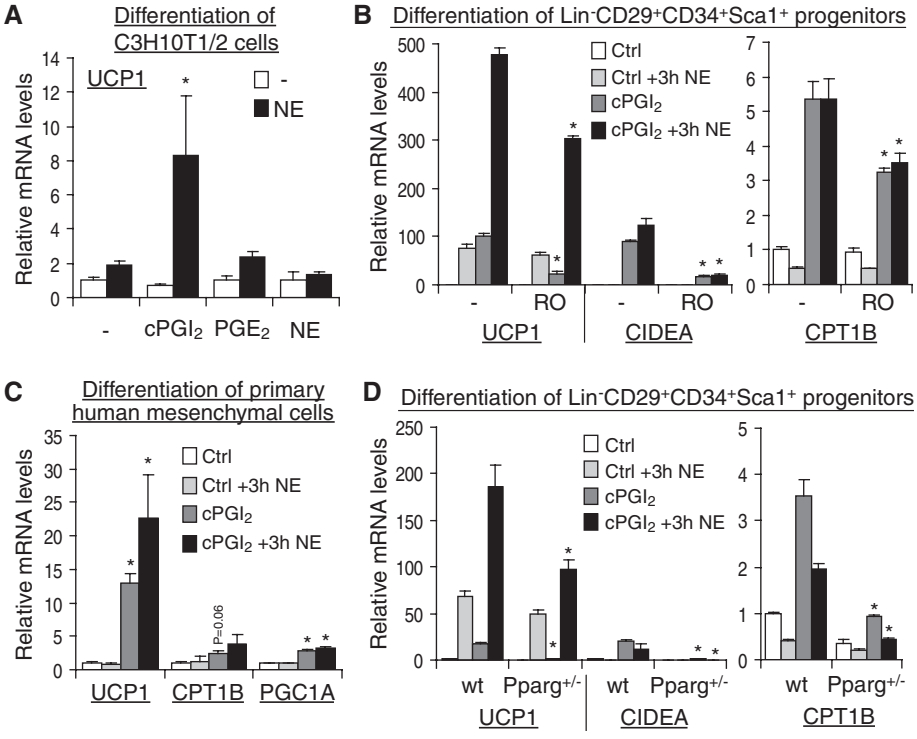

Fig. 3. Carbaprostacyclin ($cPGI_2$) shifts the differentiation of WAT mesenchymal progenitors toward a brown adipocyte phenotype. (**A** to **D**) Quantitative RT-PCR mRNA expression analysis. (A) C3H10T1/2 cells were induced to differentiate with adipogenic medium for 12 days in the presence of 1 μM of the indicated substance. Three hours before harvest, cells were acutely stimulated with 1 μM NE or vehicle in the absence of PG ($n = 3$; ANOVA interaction: UCP1 $P = 0.002$, PGC1A $P = 0.002$; *, post tests – versus $cPGI_2$: UCP1 $P < 0.01$, PGC1A $P < 0.05$). (B) Mouse $Lin^-CD29^+CD34^+Sca1^+$ mesenchymal progenitor cells were sorted from WAT-derived SVF and induced to differentiate as in (A) +/– 1 μM Ptgir antagonist RO1138452 (RO) ($n = 3$; *, $cPGI_2$-treated – versus RO $P < 0.03$). (C) Human enriched adipose tissue mesenchymal cells were treated as in (A) ($n = 3$; ANOVA interaction: $P > 0.05$; *, post tests Ctrl versus $cPGI_2$: $P < 0.001$). (D) $Lin^-CD29^+CD34^+Sca1^+$ cells from wt and $Pparg^{+/-}$ WAT were treated as in (A) ($n = 3$; *, $cPGI_2$-treated wt versus $Pparg^{+/-}$ $P < 0.015$). Means ± SEM.

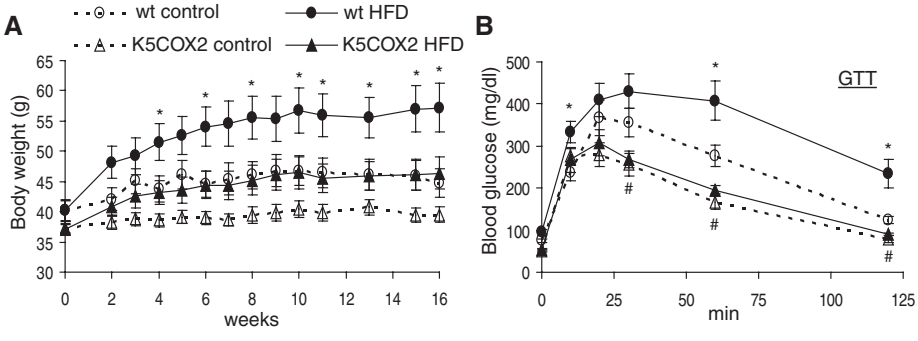

Fig. 4. Chronic COX-2 excess in WAT protects mice from diet-induced obesity and dysregulated glucose homeostasis. (**A**) Body weight time course of 7-month-old wt and K5COX2 mice fed a HFD or a control diet (60% and 10% calories from fat, respectively) for 16 weeks ($n \geq 9$, ANOVA post tests, *$P < 0.05$). (**B**) Intraperitoneal glucose tolerance test (GTT) in same mice as in (A) [$n = 7$; ANOVA post tests, wt: control versus HFD $P < 0.05$ (*); control diet: wt versus K5COX2 $P < 0.05$ (#)]. Means ± SEM.

K5COX2 mice displayed a 20% reduction in body weight, correlating with a severe reduction in body fat content but not muscle mass or bone length (Fig. 2, C and D, and fig. S16), which was reversed in animals on a celecoxib diet (fig. S17). The reduced adiposity of K5COX2 mice could not be explained by decreased food intake (Fig. 2E), increased activity of thermogenic and/or futile cycles in skeletal muscle (fig. S18), enhanced conBAT activity (fig. S19), or compromised skin insulation (figs. S18 to S22), but was associated with increased energy expenditure. Oxygen consumption was increased in K5COX2 mice compared with controls (Fig. 2F), which, along with an increase in body temperature (fig. S22), reflected a significant elevation of the resting metabolic rate (fig. S22). Consistent with increased substrate use, plasma free fatty acid and glycerol levels were lower in K5COX2 mice as compared with controls (fig. S23). Taken together, these results indicate that COX-2 has a critical role in indBAT development and function in WAT depots and that the COX-2-PG pathway contributes to adaptive thermogenesis and energy homeostasis.

Because the cellular origin of indBAT is currently a matter of dispute (*9*, *16*), we sought to determine the cell type responding to PG downstream of COX-2. Whereas NE treatment resulted in moderate but significant increases in UCP1, PGC1A, and PPARA mRNA expression, neither PGE_2 nor carbaprostacyclin ($cPGI_2$), a stable analog of PGI_2, induced these genes in mature adipocytes (fig. S24). In contrast to conBAT cells, induced brown adipocytes in WAT depots do not originate from common BAT/myogenic progenitors during cold exposure (*16*, *17*). Indeed, markers of conBAT progenitors, MYF5 and LHX8, were not enriched in indBAT of CL-treated or K5COX2 mice (fig. S25), substantiating the hypothesis that indBAT cells derive from unique mesenchymal progenitors residing in the SVF of WAT depots (*9*). As a model for multipotent mesenchymal progenitors, we first studied C3H10T1/2 cells (*16*, *18*) and treated them with PG or NE during adipogenic differentiation. Differentiation of these progenitors in the presence of $cPGI_2$ generated lipid-containing adipocytes with enhanced bona fide brown adipocyte capacities, as shown by a substantially increased response of thermogenic gene expression to postdifferentiation acute NE stimulation (Fig. 3A and fig. S26) (*19*). Additionally, acute treatment of primary SVF cells from WAT with PGE_2 or $cPGI_2$ led to a significant increase in UCP1 and PGC1A mRNA expression to an extent comparable to or greater than that induced by NE (fig. S27), supporting the notion that (progenitor) cells within the SVF are PG-responsive and in principle capable of upregulating BAT-specific genes.

We next isolated primary $Lin^-CD29^+CD34^+Sca1^+$ progenitor cells from WAT-derived SVF. These cells have the potential to differentiate along several mesenchymal lineages, including the lineage leading to white adipocytes (*20*) (fig. S28). Treatment with $cPGI_2$ or coculture with

CL-treated WAT explants induced the expression of UCP1 mRNA in these undifferentiated progenitor cells (figs. S29 and S30). Exposure of the $Lin^-CD29^+CD34^+Sca1^+$ cells to $cPGI_2$ during adipogenic differentiation potently elevated BAT marker gene expression and enhanced the post-differentiation responsiveness to NE (Fig. 3B and fig. S31). Similar results were also obtained with primary mesenchymal progenitors obtained from human WAT (Fig. 3C). The $cPGI_2$ effects in $Lin^-CD29^+CD34^+Sca1^+$ progenitors were blocked by loss-of-function of cellular PGI_2 receptors, Ptgir (7-transmembrane receptor) (*21*) or nuclear receptor PPARγ (*22*) (Fig. 3, B and D, and figs. S31 and S32) but were unaffected by PPARα/PPARβ/δ double knockout (fig. S33). Consistently, CL-induced indBAT recruitment was impaired in $Ptgir^{-/-}$ mice (figs. S34 to S36). Likewise, CL-induced indBAT recruitment was partially inhibited in $PPAR\gamma^{+/-}$ animals (figs. S37 and S38) (*23*) but not in $PPAR\alpha^{-/-}$ or $\beta/\delta^{-/-}$ mice (figs. S39 and S40), although the effect of PPARγ heterozygosity on UCP1 expression was compensated in the context of the intact tissue in vivo. These results demonstrate that COX-2 triggers recruitment of indBAT in WAT through a conserved PG-mediated differentiation shift of WAT mesenchymal progenitors toward the brown adipocyte phenotype using both membrane (Ptgir) and nuclear (PPARγ) receptor pathways.

Lastly, the critical role of COX-2 in the de novo recruitment of indBAT prompted us to assess the potential of this pathway to counteract adiposity and its pathophysiological consequences. Wild-type mice on a high-fat diet (HFD) showed a marked body weight gain throughout a 16-week feeding period (Fig. 4A). In contrast, weight gain was not significant in K5COX2 mice after 16 weeks on HFD; these mice reached body weight levels of wild-type mice on control diet (Fig. 4A). Moreover, K5COX2 mice were protected against HFD-induced fasting hyperglycemia (fig. S41), hyperinsulinemia (fig. S41), and glucose intolerance (Fig. 4B), suggesting that the stimulation of indBAT recruitment and energy expenditure through the COX-2-PG pathway confers protection against several adverse metabolic consequences of diet-induced obesity.

In conclusion, our data are consistent with a model in which NE released from sympathetic nerves induces COX-2 activity in WAT. We propose that downstream $PG(I_2)$/Ptgir/PPARγ signaling then shift(s) the differentiation of mesenchymal progenitors toward a brown phenotype with increased sensitivity to NE (fig. S1). This feed-forward mechanism results in the recruitment of indBAT in WAT depots, contributing to thermogenesis and systemic energy expenditure. Currently, there are no drugs available that induce BAT. β-adrenergic agonists that increase thermogenesis have been tested in humans as antiobesity drugs, but with limited success (*24*, *25*). Manipulation of COX-2/PG signaling in defined indBAT progenitors represents an alternative strategy for enhancing BAT activity that could help protect against energy surplus and body weight gain.

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26. We thank K. Hexel, A. Jones, D. Kucher, P. Kulozik, A. Lacher, F. Mattijssen, D. Metzger, A. Pohl-Arnold, A. Reimann, S. Schmitt, B. Steinbauer, M. Willershäuser, and A. E. Schwarz for experimental support; A. Rose for comments on the manuscript; J. Masferrer (Pfizer, St. Louis, USA) for providing celecoxib; and S. Narumiya (Kyoto, Japan) for Ptgir knockout mice. This work was supported by the German National Genome Research Network (grants 01GS0822 to M.K. and 01GS0869 to M.K. and M.H.) and by the Deutsche Forschungsgemeinschaft He3260/2-1, the Network Aging Research Heidelberg/Mannheim, and a Marie Curie Excellence Grant to S.H.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1186034/DC1
Materials and Methods
Figs. S1 to S41
References

16 December 2009; accepted 8 April 2010
Published online 6 May 2010;
10.1126/science.1186034
Include this information when citing this paper.

Genome-Wide Kinetics of Nucleosome Turnover Determined by Metabolic Labeling of Histones

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Nucleosome disruption and replacement are crucial activities that maintain epigenomes, but these highly dynamic processes have been difficult to study. Here, we describe a direct method for measuring nucleosome turnover dynamics genome-wide. We found that nucleosome turnover is most rapid over active gene bodies, epigenetic regulatory elements, and replication origins in *Drosophila* cells. Nucleosomes turn over faster at sites for trithorax-group than polycomb-group protein binding, suggesting that nucleosome turnover differences underlie their opposing activities and challenging models for epigenetic inheritance that rely on stability of histone marks. Our results establish a general strategy for studying nucleosome dynamics and uncover nucleosome turnover differences across the genome that are likely to have functional importance for epigenome maintenance, gene regulation, and control of DNA replication.

Nucleosome disassembly and reassembly, or turnover, is necessary for epigenome maintenance, but the mechanisms that are responsible remain unclear (*1*). One approach to this problem has been to map enrichment of the universal histone replacement variant, H3.3 (*2*–*6*), which requires complete unwrapping of DNA from around the histone core for its replication-independent deposition to occur. Genome-wide profiling of steady-state amounts of H3.3 from *Drosophila melanogaster* S2 cells indicated that nucleosome replacement occurs most prominently across transcribed regions of active genes and at promoters and binding sites of trithorax group (trxG) and polycomb group (PcG) proteins (*2*, *3*). Similar results were obtained for HeLa cells (*7*) and *Caenorhabditis elegans* embryos (*8*). A more direct approach, which can measure dynamics but is limited to yeast, is to express constitutive and inducible histone transgenes and to measure the relative incorporation of their encoded tagged histones (*9*–*11*). These studies indicated that turnover rates were high at promoters and chromatin boundary

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