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Obesity is induced in mice heterozygous for cyclooxygenase-2

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Abstract

In mice heterozygous for the cyclooxygenase-2 gene (COX-$2^{\pm}$) the body weight was enhanced by 33% as compared to homozygous COX-$2^{-/-}$ mice. The weights of the gonadal fat pads in COX-$2^{\pm}$ mice were enhanced by 3.5 to 4.7 fold as compared to COX-$2^{-/-}$ mice and by 1.5 to 3.5 fold as compared to wild-type controls$^{+/+}$. Serum leptin levels and leptin release by cultured adipose tissue of COX-$2^{\pm}$ mice were both elevated as compared to either control or COX-$2^{-/-}$ animals. The basal release of PGE_2 or 6 keto $PGF_{1\alpha}$ per fat pad over a 24 h incubation of adipose tissue was reduced by 80% and 95% respectively in tissue from COX-$2^{-/-}$ mice. NS-398, a specific COX-2 inhibitor, inhibited leptin release by 27% in adipose tissue from control mice, 31% in tissue from COX-$1^{-/-}$ mice and by 23% in tissue from COX-$2^{\pm}$ mice while having no effect on leptin release by adipose tissue from COX-$2^{-/-}$ mice. These data indicate that heterozygous COX-2 mice develop obesity which is not secondary to a defect in leptin release by adipose tissue.

Keywords: Adipose tissue; prostaglandin E_2; prostacyclin; prostanoids; leptin

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1. Introduction

Obesity is readily produced in animals by damage to the ventromedial nucleus or the paraventicular nucleus in the hypothalamus. Most of these effects are mediated through neuropeptides and monoamines that modulate food intake. Leptin is a key hormone involved in regulation of obesity in mice since the lack of the full-length leptin receptor or a defect in leptin production by adipose tissue can result in marked obesity. The sympathetic nervous system is also very important since low sympathetic activity is associated with obesity [1].

In 1975 Curtis-Prior [2] postulated that metabolic obesity might result from overproduction of prostaglandins by adipose tissue resulting in impaired lipolysis. However, subsequent studies by Kather et al. [3] and Curtis-Prior et al. [4] cast doubt on this hypothesis. The prostaglandin endoperoxide H synthase-2 commonly known as cyclooxygenase-2 (COX-2) is expressed at very low levels, but is induced in many cells by proinflammatory stimuli [5–9]. In contrast its isoform COX-1 is constitutively expressed in many cells. In male mice, deletion of COX-1 appeared to reduce prostaglandin levels by 99% but with minimal health effects while COX-2 null mice develop nephropathy [5–9]. However, deletion of either COX-1 or COX-2 had comparable effects in reducing intestinal as well as skin tumorigenesis and reduced tumorigenesis was also reported in mice heterozygous for COX-2 [9].

The majority of mice heterozygous for glucose transporter 4 (GLUT4) develop diabetes while this is not seen in mice homozygous for GLUT-4 [10]. The present report indicates a similar finding with regard to obesity which is seen in mice heterozygous for COX-2 but not in mice homozygous for COX-2.

2. Materials and methods

2.1. Animals

The COX-2 gene heterozygous (COX-$2^{\pm}$), homozygous (COX-$1^{-/-}$ and COX-$2^{-/-}$) knockout-mice were derived from C57/B6 × 129 Ola founders obtained from Dr. Scott Morham of the University of North Carolina [5,6]. Strain C57/DBA1 of COX-1-deficient, COX-2-deficient, and wild type (+/+) mice used in these studies was developed at the Memphis Veterans Affairs Medical Center. The strain was created by back-crossing C57BL/J6 COX-deficient mice with WT DBA/1 mice for six generations followed by extensive intercrossing. Adult mice of this strain did not exhibit reduced longevity or the severe renal pathology described for the original COX-2-deficient C57BL/J6 strain [6,7,9]. The COX-2 (−/−) and COX-1 (−/−) mice were obtained by breeding (−/−) males to (±) females because of the infertility of the COX-2 (−/−) and COX-1 (−/−) females. Animals were housed in group Plexiglas cages at 25°C ± 1°C and kept on a 12-h light/12-h dark cycle. Food and water were available ad libitum. All experimental mice were offspring of a number of simultaneous matings. The experiments were performed by using animals of genotypes COX-1- and COX-2-deficient homozygotes, COX-2-heterozygotes and their wild-type control littermates. The genotype of each animal was determined by PCR as described

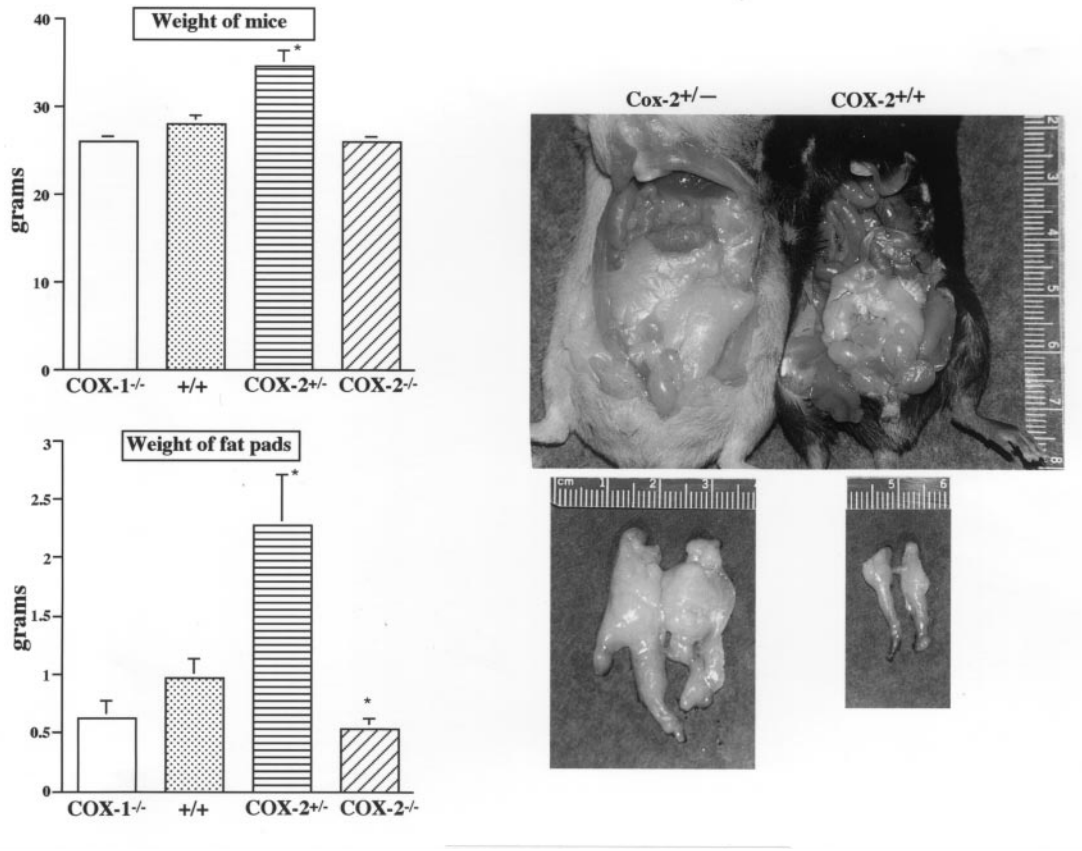

Fig. 1. Comparison of body weight and gonadal fat pad weight between normal, COX-$1^{-/-}$, COX-$2^{+/-}$ and COX-$2^{-/-}$ mice. The *right* side of the figure shows the epididymal fat pads from a control (COX-$2^{+/+}$ mouse versus a COX-$2^{+/-}$ mouse. The values in the Left side panels are the means ± SEM of animal weight or epididymal weight among control and COX knockouts and are for 6 animals in each group. Significant differences from the controls$^{+/+}$ are indicated by an asterisk ($p < 0.05$).

by Ballou et al. [11]. There was no detectable COX-2 mRNA in the spinal cord or pawpads of COX-2 (−/−) mice or of COX-1 mRNA in tissues from COX-1 (−/−) mice [11].

Mice (9–12 weeks of age) were fed for 5 weeks a pelleted high fat and high sugar diet containing 27% casein, 20% Crisco, 46% sucrose, 2% RP vitamin mix and 5% RP mineral mix #10 that was supplied by Purina Mills of Richmond, IN. The mice were fed this diet because in preliminary studies we found little release of leptin by adipose tissue from COX-2 −/− mice fed laboratory chow since they had less than 300 mg of epididymal adipose tissue per animal.

The epididymal or parametrial adipose tissue for each experiment was obtained from a single mouse. Approximately equal numbers of male and female mice were utilized for the experiments shown in Figs. 1-3 while the data in Figs. 4 and 5 are for male mice. The adipose tissue was weighed prior to being cut into small pieces which were added to 50 ml plastic tubes containing 5 ml of incubation buffer. The adipose tissue pieces were isolated and incubated under aseptic conditions. The amount of tissue incubated in each tube was as

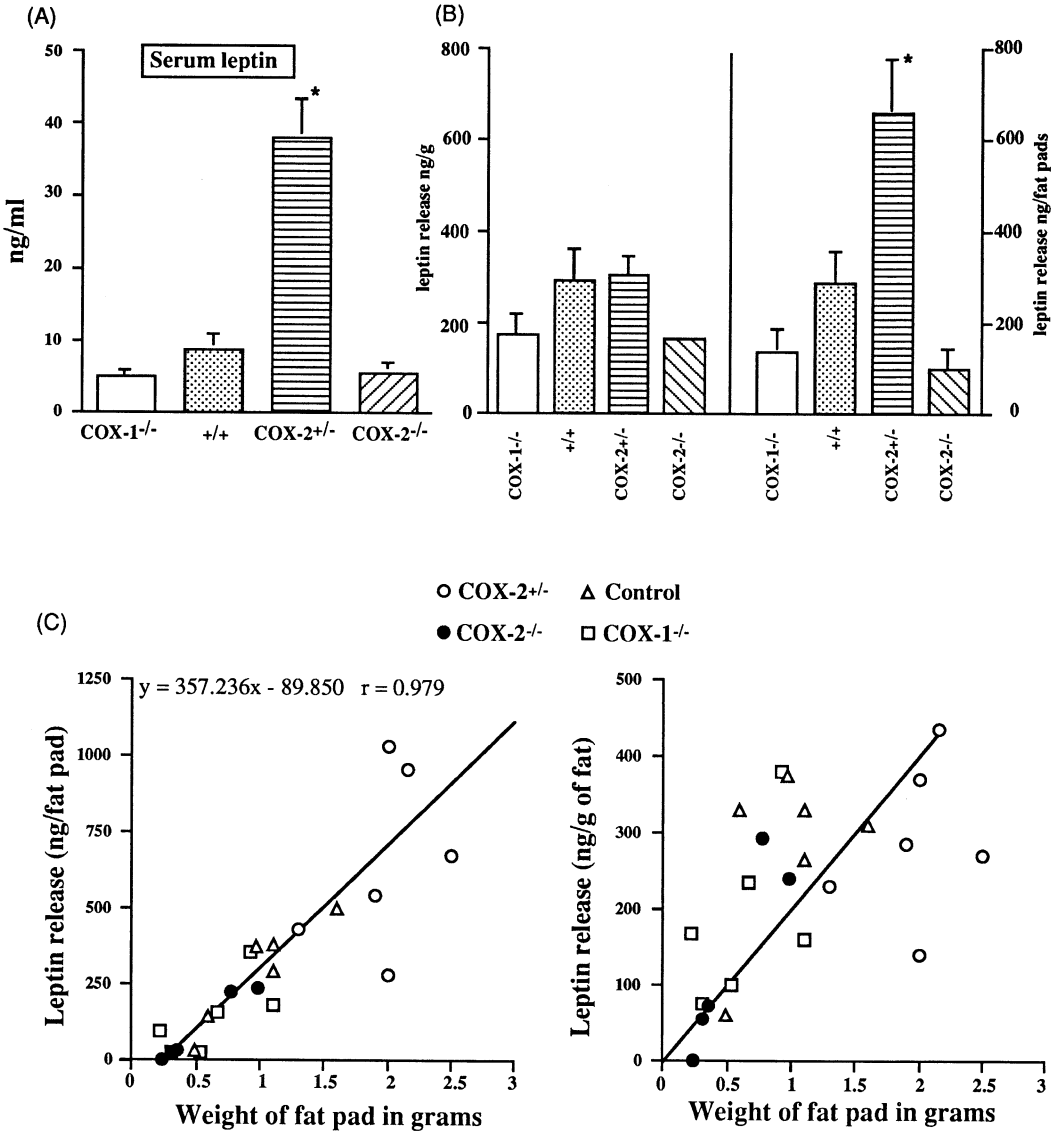

Fig. 2. Serum leptin levels in COX transgenic mice and leptin release by adipose tissue incubated in primary culture. Panels A and B, represent serum leptin in mice and the release of leptin by adipose tissue explants into the medium over 24 h incubation in medium containing 25 nM dexamethasone. The values are the means ± SEM of 5–6 experimental replications using tissue from the mice whose body weight and gonadal fat weight are shown in Fig. 1. Panel C shows the data for leptin release per g of fat and per total amount of gonadal fat from the individual experiments in panels A and B plotted against individual fat pad weight. The correlation coefficient between total leptin release per gonadal fat pads and weight of fat pads was 0.979.

follows in mg: 80 for COX-$2^{-/-}$, 90 for COX-$1^{-/-}$, 130 for controls$^{+/+}$ and 200 for COX-$2^{\pm}$ for the studies shown in Figs. 2 and 3. The buffer for incubation of adipose tissue was DMEM/Ham's F12 (1:1), as modified by Fain et al. [12].

All agents were added at the start of the incubation. Aliquots of the medium were taken

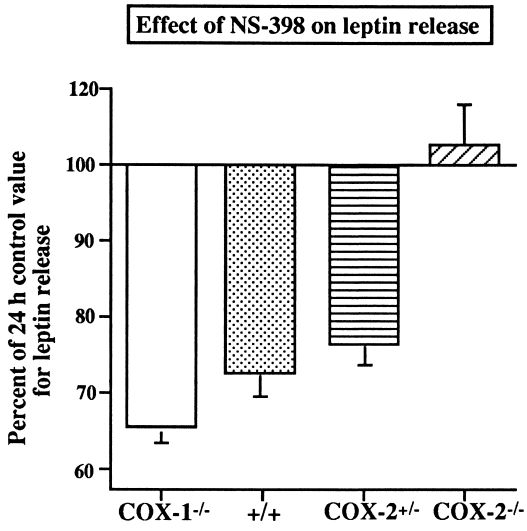

Fig. 3. Effects of NS-398 a cyclooxygenase-2 inhibitor on leptin release by fat pads. Pieces of adipose tissue were incubated for 24 h in 5 ml of medium in the presence of 25 nM dexamethasone either without or with 1 μM NS 398. The effects of NS-398 are given as percentage ± SEM of the leptin release over 24 h in the absence of NS-398. The data are from the same experiments shown in Fig. 2 where the data for leptin release in the absence of NS-398 are shown.

at 24 h and stored at –20°C for measurement of leptin. The leptin content of 20 to 50 μl aliquots of the incubation medium was determined using radioimmunoassay kits from Linco Research, Inc. Blood glucose levels were determined using the One-Touch system (LifeScan, Milipitas, CA). Samples for glucose determination were taken in the early afternoon by a single cut of the mouse tail. Serum insulin was measured using a radioimmunoassay kit from Linco Research designed for analysis of rodent insulin. PGE_2 and 6 keto $PGF_{1\alpha}$ were measured by radioimmunoassays as described by Leffler and Busija [13].

The effects of added agents were calculated as the percentage change from the incubation control in each experiment since this resulted in a more normal distribution of the data. Statistical comparisons were made using Student's *t* test on the paired differences.

3. Results

The data in Fig. 1 demonstrate that in 15 week old COX-$2^{\pm}$ mice fed a diet high in fat and sucrose for 5 weeks the weight of the mice was 24% greater than that of controls (+/+) of the same age fed the same diet for the same period of time (*Left Panels*). The weight of the COX-$2^{\pm}$ mice was 34% greater than that of COX-$2^{-/-}$ mice. Even greater differences were seen in the weight of the fat pads (Fig. 1, *Right Panels*) which were 134% greater in COX-$2^{\pm}$ mice than in controls +/+ and 328% greater than of COX-$2^{-/-}$ mice (Fig. 1).

The obesity of the COX-$2^{\pm}$ mice was not due to a defect in leptin biosynthesis since the serum leptin content was elevated by 336% as compared to that of controls (Fig. 2A). Similar

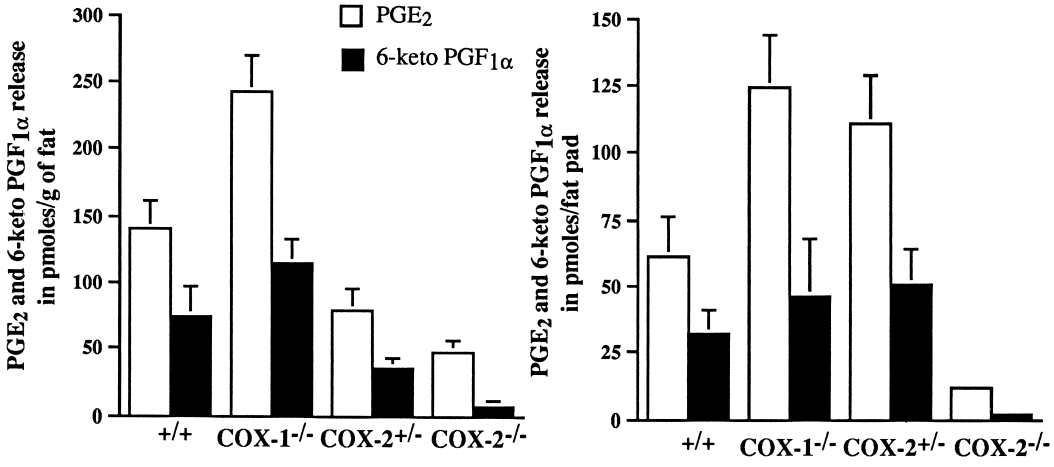

Fig. 4. Prostaglandin E_2 (PGE_2) and 6-keto $PGF_{1\alpha}$ (PGI_2) release by fat pads. Pieces of adipose tissue were incubated for 24 h. The values for PGE_2 and for 6-keto $PGF_{1\alpha}$ release to the medium are the means ± SEM [four mice in each group]. The total amount of epididymal adipose tissue in each group averaged 0.5 ± .3 g in COX-$1^{-/-}$ mice, 0.4 ± 0.1 g in control +/+ mice, 0.3 ±0.1 in tissue from COX-$2^{-/-}$ mice and 1.4 ± 0.1 g in tissue from COX-$2^{+/-}$ mice as the mean ± SEM.

results were seen with regard to leptin release by pieces of white adipose tissue incubated in primary culture for 24 h in the presence of 25 nM dexamethasone (Fig. 2B). The enhanced release of leptin was especially evident when expressed per total fat pad rather than as release per g of fat. These in vitro studies were done in the presence of 25 nM dexamethasone which enhances leptin release by pieces of mouse adipose tissue over a 24 h incubation in primary culture [12]. It is well established that dexamethasone inhibits the induction of COX-2 in many cells in response to proinflammatory agents [14,15], but we found a nonsignificant 4% inhibition of PGE_2 formation when 25 nM dexamethasone was present if mouse adipose tissue was incubated for 24 h [12].

The data for leptin release were quite variable especially in the COX-$2^{\pm}$ mice. Leptin release (total) by the fat pads from each mouse is plotted in Fig. 2C versus fat pad weight. Most of the variation in leptin release was related to the amount of adipose tissue per mouse. When total leptin release for all groups was plotted versus amount of gonadal fat all the values fit to a line with a correlation coefficient of 0.98 (Fig. 2C).

The effect of the cyclooxygenase-2 selective inhibitor NS-398 [16–17] on leptin release was examined in the presence of 25 nM dexamethasone (Fig. 3). As expected, if endogenous prostanoids contribute to leptin release, there was a 23–31% inhibition by NS-398 of leptin release by adipose tissue from all groups except the COX-$2^{-/-}$ mice. The lack of an effect of NS-398 on leptin release in COX-$2^{-/-}$ mice supports the assumption that NS-398 is a specific inhibitor of COX-2.

The endoperoxide synthase activity of the COX enzymes converts arachidonic acid to PGG_2 and the peroxidase activity of the COX enzymes converts PGG_2 to PGH_2. PGH_2 is isomerized into PGE_2 or is converted to PGI_2 via prostacyclin synthase. Therefore, we measured the formation of PGE_2 and PGI_2 based on the accumulation of 6-keto $PGF_{1\alpha}$ which

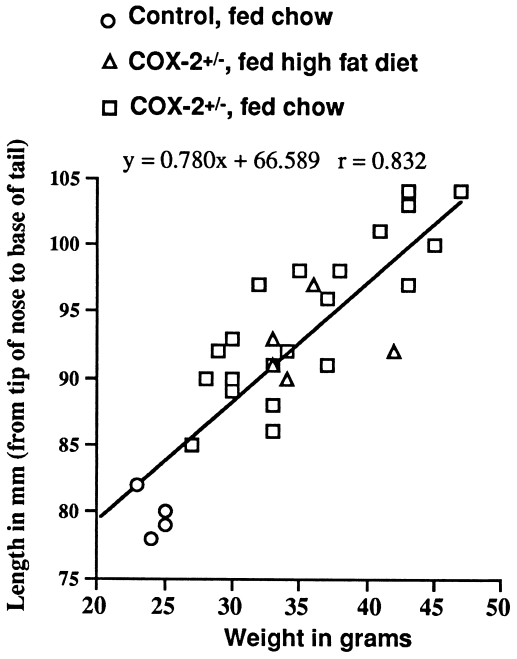

Fig. 5. Correlation between body weight and length in control versus COX-2 (+/−) male mice. The individual data are plotted for 4 control (+/+) mice shown by circles, 6 COX-$2^{+/-}$ mice fed the synthetic diet with 20% fat for 6 weeks shown by triangles and 22 COX-$2^{+/-}$ mice fed breeder chow with 10% fat shown by squares. The age of the mice at the end of the experiments ranged from 13–14 weeks. The correlation coefficient for length versus total weight was 0.832.

is the degradation product of PGI_2. PGE_2 release to the medium from explants of adipose tissue that were cultured for 24 h was 141 pmol/g of fat from control mice, while 6-keto $PGF_{1\alpha}$ was about 38% of PGE_2 accumulation (Fig. 4). The formation of PGE_2 and 6-keto $PGF_{1\alpha}$ in epididymal fat pads of COX-$1^{-/-}$ mice were 2 and 1.4-fold higher than in control mice, respectively. In COX-$2^{\pm}$ mice, the release of PGE_2 and 6-keto $PGF_{1\alpha}$ were comparable to that of COX-$1^{-/-}$, in that they were 2- and 1.6-fold higher than control mice. In COX-$2^{-/-}$ mice, the total release of PGE_2 by the epididymal fat pads was reduced to 20% of the value for control mice, while 6-keto $PGF_{1\alpha}$ production was 5% of the value for control mice. These data are in agreement with the finding that prostacyclin formation is impaired in COX-$2^{-/-}$ mice and this prostanoid has been reported to be responsible for the impaired embryo implantation seen in COX-$2^{-/-}$ mice [17].

We also measured the COX activity of homogenates of mouse adipose tissue [pooled adipose tissue from 4 mice for each group] incubated in the presence of 20 μM arachidonic acid. The COX activity was increased after a 24 h incubation of adipose tissue from a zero-time activity of 2.3 pmoles of PGE_2 formed over 30 min per g of adipose tissue to a value of 3.2 pmoles in tissue from control +/+ mice. The COX activity of homogenates of tissue from COX-$1^{-/-}$ mice after a 24 h incubation was identical to that of controls. The COX activity of homogenates from COX-$2^{\pm}$ mice was 4.1 while that of homogenates from COX-$2^{-/-}$ mice was below the sensitivity of the assay (less than 0.05). These data agree with those obtained

by measuring cumulative prostanoid accumulation in the medium over a 24 h incubation of explants of adipose tissue from the same mice (Fig. 4).

NS-398 (1μM) was used to determine the contribution of COX-1 and COX-2 activities to PGE_2 formation and release. NS-398 reduced the release of PGE_2 by adipose tissue from COX-$1^{-/-}$ mice over a 24 h incubation from 315 ± 215 pmol PGE_2 g of fat to undetectable levels (3 ± 4 pmol PGE_2 /g of fat) as the mean ± SEM of five experiments. The release of PGE_2 by adipose tissue from COX-$2^{-/-}$ mice was 21 ± 12 pmol/g in the presence of NS-398 and 31 ± 20 pmol/g of fat in its absence. In these experiments the formation of PGE_2 over 24 h by adipose tissue from control (+/+) mice was 282 ± 140 in the absence and 61 ± 40 in the presence of NS-398. The data indicate that in gonadal fat the formation of PGE_2 which is the result of cyclooxygenase-1 activity is between 20 to 60 pmol/g over a 24 h incubation.

We wondered whether the development of obesity seen at 14 weeks in COX-$2^{\pm}$ mice required 5 weeks exposure to the diet high in fat and sucrose. However, we found the same increase in the weight of COX-$2^{\pm}$ mice fed mouse breeder chow (a complex diet with 10% fat) as in mice fed a high fat diet since they were 7–8 weeks of age (Fig. 5). We also kept some mice on the diets for an additional 14 weeks. At the end of 28 weeks the weight of the COX-$2^{\pm}$ mice fed breeder chow was 46 ± 2 g (mean ± SEM, $n = 4$) while the weight of the COX-$2^{\pm}$ mice fed the synthetic high fat diet was 45 ± 2 g (mean ± SEM, $n = 4$). However, the weight of the epididymal fat pads averaged 2.9 ± 0.1 g in the mice fed the high fat diet as compared to 1.5 ± 0.2 g in mice fed the breeder chow when these mice were sacrificed at 28 weeks.

The data in Fig. 5 indicate that the increase in body weight of the COX-$2^{\pm}$ mice was accompanied by an increase in body length. Clearly the increase in weight of the COX-$2^{\pm}$ mice involves more than just accumulation of body fat.

The obesity seen in COX-$2^{\pm}$ mice at 18–20 weeks of age was not accompanied by hyperglycemia since the blood glucose value averaged 105 ± 2 mg/dl in six COX-$2^{\pm}$ mice (18 wks of age fed a high fat diet for 10 wks) as compared to 120 ± 6 mg/dl in ten 14 to 20 week old COX-$2^{\pm}$ mice fed breeder chow and 108 ± 7 mg/dl in four control (+/+) mice (13 weeks of age). We also measured blood glucose in COX-$2^{\pm}$ mice at 28 weeks of age that had been fed the high fat diet for 20–21 weeks and found a value of 116 ± 6 mg/dl (mean ± SEM, $n = 4$). The serum insulin values for the mice shown in Fig. 1 were as follows: 0.3 ± 0.1 nM for COX-$2^{-/-}$ mice, 0.6 ± 0.2 nM in COX-$2^{\pm}$ mice, 0.2 ± 0.1 nM in COX-$1^{-/-}$ mice and 0.2 ± 0.1 nM in control mice. These data indicate that the COX-$2^{\pm}$ mice have no significant elevations of blood glucose but have variable increases in serum insulin.

4. Discussion

Several reports have appeared on the effects of knockout mutations of the COX-2 gene [6–9,11,18–20]. Two of these reports have mentioned changes in mice heterozygous for COX-2. Oshima et al. [19] noted that the formation of intestinal polyps was reduced from 652 per control$^{+/+}$ mouse to 224 in COX-$2^{\pm}$ mice as compared to 93 in COX-$2^{-/-}$ mice. Li et al. [20] found that the febrile response to lipopolysaccharide administration was abolished in COX-$2^{\pm}$ as well as COX-$2^{-/-}$ mice.

In rat adipose tissue, exogenous PGE_2 stimulates leptin release [21]. The addition of PGE_2

to cut pieces of mouse adipose tissue incubated in primary culture for 24 h results in a 30–40% increase in leptin release [12]. The data in Fig. 3 suggest that one-fourth of the total release of leptin by adipose tissue from mice incubated in the presence of dexamethasone might involve prostanoids formed via COX-2 based on studies with NS-398. However, there are clearly compensatory mechanisms since in mice that do not express any COX-2 there appeared to be leptin release appropriate to the amount of gonadal fat (Fig. 2). There was no inhibitory effect of deletion of COX-1 on total PGE_2 or PGI_2 (measured as 6 keto $PGF_{1\alpha}$) formation by adipose tissue (Fig. 4). Furthermore, only a small quantity of PGE_2 was formed by adipose tissue from COX-$2^{-/-}$ mice which supports the hypothesis that most of the PGE_2 formed by explants of mouse adipose tissue in primary culture after a 24 h incubation is the result of COX-2 activity. PGI_2 formation in $COX2^{-/-}$ was also impaired because $PGH_{2,}$ the precursor for PGI_2 as well as $PGE_{2,}$ is formed by the COX enzymes.

Another role for PGE_2 in mouse adipose tissue involves the regulation of lipolysis [12]. Near maximal inhibition of COX-2 by NS-398 caused a 30% increase in basal lipolysis over a 24 h incubation [12]. Therefore, endogenous prostanoids appear to inhibit lipolysis and stimulate leptin release in mouse adipose tissue (Fig. 3).

The data in Fig. 5 show that not only the weight but the length of the mice (tip of nose to base of tail) was greater in the COX-$2^{\pm}$ mice. Similar increases in fat content and length without elevations in blood glucose have been seen in mice overexpressing the GH-releasing hormone (GHRH) [22]. We measured the level of GH in plasma of fed COX-$2^{\pm}$ mice but found normal values for GH (24 $\pm$ 8 ng/ml of GH as the mean $\pm$ SEM for 11 mice in unpublished studies of J.N. Fain and J.F. Hyde). In contrast fed mice with the hGHRH transgene have values of around 300 ng/ml of GH [22]. However the mice overexpressing the hGHRH gene are comparable in other respects to the COX-$2^{\pm}$ mice in demonstrating normal blood glucose values, elevated blood insulin and leptin, enhanced body length and over a 100% increase in abdominal fat depots [22].

There are other mouse obesity mutants [23] but the normal coat color rules out the agouti/yellow obese (*AY*) while the normal leptin levels eliminate the *ob* mutant. The lack of hyperglycemia and diabetes would appear to eliminate the *db* mutation which is due to a defect in the long form of the leptin receptor [23]. The serotonin receptor type-2C null mutant mouse has late onset obesity with hyperinsulinemia [24] which is not seen until after 18 weeks of age. In contrast we saw a 29% increase in body weight of COX-$2^{\pm}$ mice at 9 weeks of age in mice fed ad libitum on laboratory chow.

Obesity is also seen in the NYP5 receptor knockout mouse that is delayed in onset with little effect being seen in the first 20 weeks [25]. The injection of gold thioglucose results in obesity within 14 days after its injection secondary to a reduction in leptin receptors in the hypothalamus [26]. The sequence of events was a rise in serum insulin at 1 week post injection followed by a doubling of gonadal fat and serum leptin at 2 weeks but no hyperglycemia until 4 weeks [26]. Over-expression of the human Axl tyrosine kinase receptor in myeloid cells of mice also results in early onset (8 weeks) obesity but it is accompanied by marked hyperglycemia [27]. In summary the COX-$2^{\pm}$ mice have a unique type of obesity that is most comparable to that of mice with elevated levels of GH due to over-expression of GHRH.

While the release of leptin is attributed to the adipocytes in adipose tissue the same is not

necessarily so for PGI_2 and PGE_2 formation by explants of adipose tissue in primary culture. It is probable that the majority of the prostanoids released to the medium by explants of adipose tissue incubated in primary culture for 24 h (Fig. 4) are derived from the non-fat cells present in adipose tissue [28,29]. If the release of PGE_2 and of 6-keto $PGF_{1\alpha}$ by explants of human adipose tissue was compared to that by isolated human adipocytes over a 48 h incubation the rate of release by adipocytes was less than 10% of that by explants of intact tissue (unpublished results, Fain and Bahouth). These findings suggest that the effects of NS-398 on leptin release (Fig. 3) and lipolysis [12] by adipose tissue reflect inhibition of the release of prostanoids by endothelial and/or smooth muscle cells comprising the vascular network in adipose tissue which then react with prostanoid receptors on the surface of nearby adipocytes.

Obesity in COX-$2^{\pm}$ mice was not accompanied by diabetes which makes this model more comparable to the majority of humans who have obesity without diabetes. The lack of obesity in COX-$2^{-/-}$ mice may be the result of compensating mechanisms that are only activated in the total absence of COX-2. A similar situation is seen in mice where complete deletion of GLUT-4 does not result in diabetes while in mice expressing only one allele a significant fraction of the mice develop diabetes [10]. The finding that obesity can be seen after deletion of one allele of the COX-2 gene suggests that the role of PGE_2 or related prostanoids in obesity should be given serious consideration. It is probable that the obesity seen in the COX-$2^{\pm}$ mice is the result of prenatal developmental changes in the centers controlling food intake and metabolism. Furthermore, the gene dosage produced by these prenatal developmental changes in COX-2 expression appear to determine the obesity phenotype. In COX-$2^{-/-}$ animal, obesity was not observed because the gene dosages were altered in such a way to restore the balance between food intake and metabolism while in COX-$2^{\pm}$ animals a disparity between these genes might be the primary cause for obesity.

Acknowledgments

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