Intestinal alkaline phosphatase prevents metabolic syndrome in mice

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Metabolic syndrome comprises a cluster of related disorders that includes obesity, glucose intolerance, insulin resistance, dyslipidemia, and fatty liver. Recently, gut-derived chronic endotoxemia has been identified as a primary mediator for triggering the low-grade inflammation responsible for the development of metabolic syndrome. In the present study we examined the role of the small intestinal brush-border enzyme, intestinal alkaline phosphatase (IAP), in preventing a high-fat-diet–induced metabolic syndrome in mice. We found that both endogenous and orally supplemented IAP inhibits absorption of endotoxin (lipopolysaccharides) that occurs with dietary fat, and oral IAP supplementation prevents as well as reverses metabolic syndrome. Furthermore, IAP supplementation improves the lipid profile in mice fed a standard, low-fat chow diet. These results point to a potentially unique therapy against metabolic syndrome in at-risk humans.

atherosclerosis | cytokines | diabetes | dysbiosis | steatosis

Metabolic syndrome is a complex syndrome composed of a cluster of disorders that includes obesity, glucose intolerance, insulin resistance, abnormal lipid profile (dyslipidemia), fatty liver, and hypertension (1, 2). Metabolic syndrome leads to type 2 diabetes, atherosclerosis, and nonalcoholic fatty liver disease (1, 2). Approximately 35–39% of the US population suffers from the syndrome (3). This epidemic of metabolic syndrome has devastating consequences in terms of mortality, morbidity, and total healthcare expenditures (4).

Recently, "metabolic endotoxemia" has been proposed to be central to the pathogenesis of metabolic syndrome. The Gram-negative bacterial cell wall component lipopolysaccharide (LPS) is known as endotoxin, and metabolic endotoxemia is defined as a two- to threefold persistent increase in circulating endotoxin concentrations above the normal levels (5). Metabolic endotoxemia leads to low-grade systemic inflammation as evidenced by increased serum levels of tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, and IL-6 (5). It is well recognized that chronic inflammation causes damage to pancreatic beta cells (6), hepatocytes (7), and vascular endothelial cells (8), and dysfunction of these cells is thought to contribute to metabolic syndrome.

A high-fat diet (HFD) has been shown to cause metabolic endotoxemia in animals and humans (5, 9), but the underlying molecular mechanisms remain incompletely understood. Ghoshal et al. (10) demonstrated that intestinal epithelial cells (enterocytes) internalize LPS from the apical surface, which is then transported to the Golgi apparatus where it complexes with chylomicrons, the lipoproteins that transport the absorbed long-chain fatty acids in enterocytes. The chylomicron–LPS complex is then secreted into mesenteric lymph and makes its way into the systemic circulation. Excess chylomicron formation during high-fat feeding leads to prolonged chylomicronemia (complexed with LPS) that ultimately induces systemic inflammation. Also, it has been shown that an HFD causes local intestinal inflammation (11). Systemic and local inflammation lead to overexpression of proinflammatory cytokines (12), which cause increased gut permeability (13) and an acceleration of endotoxin translocation (14), resulting in a vicious cycle of endotoxemia. A central role of LPS in the pathogenesis of metabolic syndrome is also supported by the observation that mice lacking toll-like receptor 4 (TLR4), the receptor for LPS, are resistant to HFD-induced inflammation, obesity, and insulin resistance (15).

We and others have shown that the brush-border enzyme intestinal alkaline phosphatase (IAP) detoxifies a variety of bacterial toxins, including LPS, CpG DNA, and flagellin (16). Furthermore, it has been reported that inhibition of endogenous IAP by L-phenylalanine (Phe) increases serum endotoxin levels (17). Based upon these previous observations regarding IAP function, we hypothesized that this enzyme could play an important role in preventing gut-derived systemic inflammation. Here we report that endogenous IAP plays a critical role in reducing endotoxemia, and oral supplementation with IAP prevents HFD-induced endotoxemia, as well as metabolic syndrome in mice. We also show that IAP supplementation was able to reverse HFD-induced metabolic syndrome and improves the lipid profile in mice fed a standard low-fat chow diet (LFD). Taken together, these findings suggest that oral IAP supplementation may represent a unique therapeutic approach for the prevention or treatment of metabolic syndrome in humans.

Results

IAP Prevents Endotoxemia. Given that IAP detoxifies LPS and other bacterial toxins, we predicted that IAP knockout (KO) mice (18) would suffer from metabolic endotoxemia and also metabolic syndrome. Indeed, we found that IAP-KO mice suffer from endotoxemia (Fig. 1*A*) as well as overexpression of the proinflammatory cytokines TNF-α (Fig. 1*B*) and interleukin-1β (IL-1β) (Fig. S1*A*) IAP-KO mice also had greatly increased gut permeability based on enhanced absorption of orally administered dextran-FITC (Fig. 1*C*). As expected, this increased gut permeability caused enhanced LPS translocation in IAP-KO mice compared with WT mice (Fig. 1*D*).

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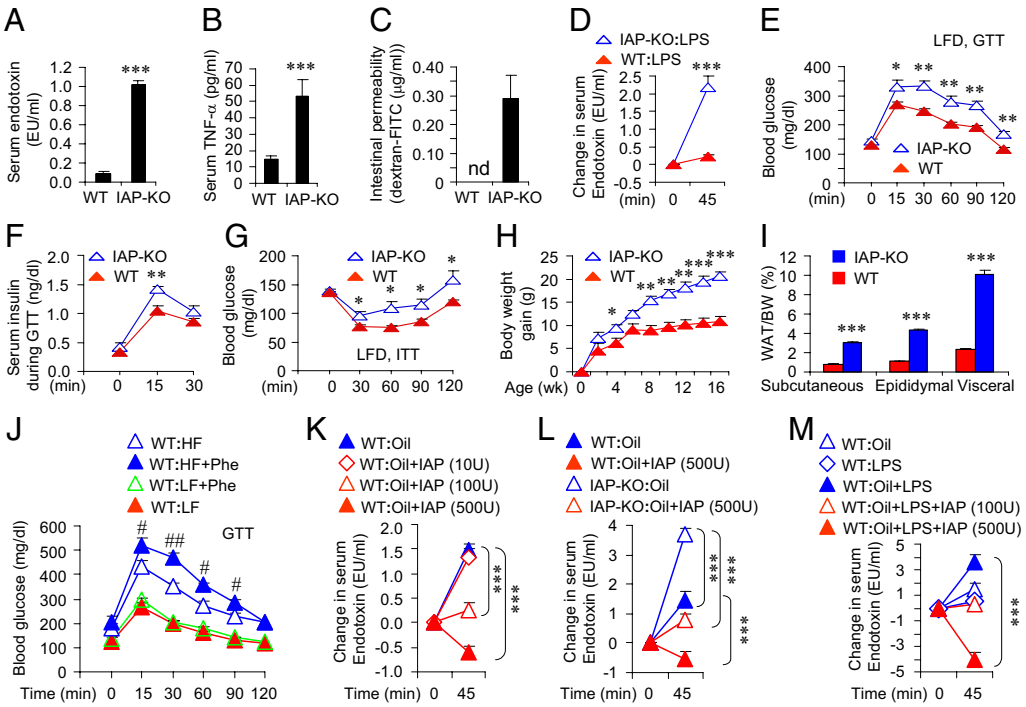

Fig. 1. IAP prevents endotoxemia. Groups of 4- to 17-wk-old C57BL/6 male IAP-KO (*Akp3*$^{-/-}$) mice and their WT littermates (n = 5–10 each group) were used in these experiments unless otherwise indicated (*SI Materials and Methods*). Mice received a low-fat diet (LFD, 14% kcal from fat) unless otherwise indicated. (*A*) Serum endotoxin levels. (*B*) Serum TNF-α levels. (*C*) Intestinal permeability as determined by quantifying the amount of FITC-dextran (70 kDa) levels in the serum after its oral gavage. (*D*) Change in serum endotoxin levels after oral LPS gavage. (*E*) Blood glucose levels during glucose tolerance test (GTT). (*F*) Serum insulin levels during the first 30 min of GTT. (*G*) Blood glucose levels during insulin tolerance test (ITT). (*H*) Body weight gain by mice on LFD. (*I*) White adipose tissue (WAT) distribution. (*J*) Blood glucose levels during GTT in mice receiving LFD (LF group) or high-fat diet (HFD, 45% kcal from fat) (HF group) treated with L-phenylalanine (Phe, 10 mM in the drinking water), a specific inhibitor of IAP. (*K*) Inhibition of corn-oil–induced endotoxemia in CD-1 mice by IAP in a dose-dependent manner. (*L*) Inhibition of corn-oil–induced endotoxemia in WT and IAP-KO mice. (*M*) Inhibition of corn-oil– plus LPS-induced endotoxemia in CD-1 mice by IAP in a dose-dependent manner. Statistics: data expressed as mean ± SEM. Two-tailed unpaired Student's t test. For multiple comparisons, analysis of variance with Tukey was used. * or $^{\#}P < 0.05$; ** or $^{\#\#}P < 0.01$; *** or $^{\#\#\#}P < 0.001$. The number sign (#) refers to the HF vs. HF + Phe comparison.

The above data established that the IAP-KO mice display evidence of metabolic endotoxemia. We next investigated whether these mice also display features of metabolic syndrome. We observed that IAP-KO mice suffer from glucose intolerance (Fig. 1*E* and Fig. S1*B*) and hyperinsulinemia (Fig. 1*F*). An insulin tolerance test (ITT) showed that IAP-KO mice had significantly higher glucose levels at multiple time points, indicating a state of insulin resistance in these animals (Fig. 1*G* and Fig. S1*C*). We also observed metabolic-syndrome–associated obesity in IAP-KO mice (Fig. 1*H*) and found that these mice accumulated higher body fat (white adipose tissue, WAT) compared with WT (Fig. 1*I*), including much more intraabdominal fat (Fig. S1*D*). These data indicate that endogenous IAP plays a critical role in preventing metabolic endotoxemia and the resultant metabolic syndrome in mice.

The amino acid Phe specifically inhibits the enzymatic activity of IAP. Mice receiving an HFD and Phe (HF + Phe group) had impaired glucose tolerance compared with mice receiving an HFD alone (HF group), indicating the preventive role of endogenous IAP (Fig. 1*J* and Fig. S1*E*). In addition, the HF + Phe group had significantly higher serum endotoxin levels (Fig. S1*F*). Interestingly, the related groups consumed the same amount of food and had similar weight gain (Fig. S1 *G* and *H*). Furthermore, IAP-KO mice receiving an HFD also suffered from type 2 diabetes as evidenced by higher fasting glucose levels and abnormal glucose tolerance tests (Fig. S1 *I* and *J*).

An HFD is associated with enhanced translocation of LPS from the gut to the systemic circulation through chylomicrons. We found higher serum LPS concentrations in WT mice fed corn oil alone compared with corn oil plus calf IAP, and these inhibitory effects of IAP were found to be dose dependent (Fig. 1*K*). We also observed higher serum LPS concentrations in IAP-KO mice fed with corn oil compared with WT mice (Fig. 1*L*). Oral IAP supplementation prevented corn-oil–induced endotoxemia in both groups of mice (Fig. 1*L*) and in a dose-dependent manner was able to reduce endotoxemia when excess LPS was administered along with the corn oil (Fig. 1*M*).

IAP Prevents Chronic High-Fat-Diet–Induced Metabolic Syndrome. We next assessed whether over a prolonged period, oral supplementation with IAP could prevent metabolic syndrome. We exposed 15-wk-old male C57BL6 mice to an HFD (45% kcal from fat) ± IAP (100 units/mL drinking water) for 11 wk. Control mice (low fat, LF group) consumed a standard chow LFD (14% kcal from fat) only. As expected, the mice receiving the HFD + IAP (HF + IAP group) exhibited better glucose tolerance (Fig. 2*A*) and less postprandial hyperinsulinemia (Fig. 2*B*) and insulin resistance (Fig. 2*C*) than mice receiving the HFD alone (HF group). Fasting blood glucose levels were higher in the HF compared with LF mice, whereas there were no differences between LF and HF + IAP groups (Fig. 2 *A* and *D* and Fig. S2 *A* and *B*). We observed higher body weight in the HF group, however no significant difference between LF and HF + IAP groups (Fig. S2*C*). The HF and HF + IAP groups had nearly equal energy intake (Fig. S2*D*); however, the feed efficiency [FE = weight gained (g)/kcal consumed × 100] was lower, although insignificant, in the HF + IAP group (Fig. S2*E*). We also found that IAP prevented HFD-induced decrease in pancreatic weight (Fig. 2*E*). Compared with

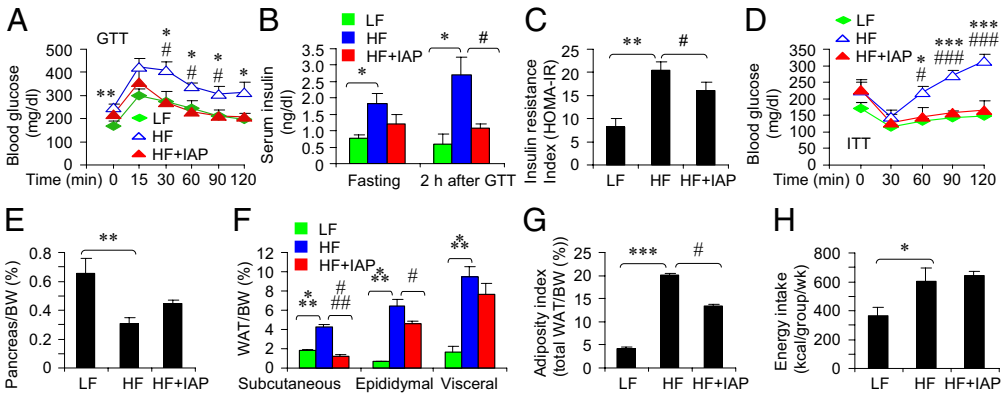

Fig. 2. IAP prevents chronic HFD-induced glucose intolerance and insulin resistance. Groups of 15-wk-old WT male C57BL/6 mice ($n = 5$ for each group) were fed an HFD (45% kcal from fat) ± IAP (100 units/mL in drinking water) for 11 wk. A control group of mice received LFD (14% kcal from fat). (*A*) Blood glucose levels during GTT in mice receiving LFD (LF group), HFD (HF group), and HFD + IAP (HF + IAP group). (*B*) Serum insulin levels during GTT. (*C*) Insulin resistance index (homeostasis model assessment of insulin resistance, HOMA-IR). (*D*) Blood glucose levels during ITT. (*E*) Weight of pancreas. (*F*) White adipose tissue (WAT) expressed as percentage of body weight (BW). (*G*) Adiposity index. (*H*) Energy intake by different groups. Statistics: data expressed as mean ± SEM. Two-tailed unpaired Student's *t* test. For multiple comparisons, analysis of variance with Tukey was used. * or $^{\#}P < 0.05$; ** or $^{\#\#}P < 0.01$; *** or $^{\#\#\#}P < 0.001$. The asterisk (*) refers to the LF vs. HF comparison and the number sign (#) refers to the HF vs. HF + IAP comparison.

the HF group, mice in the HF + IAP group also showed an overall reduction in body fat, most dramatically seen in the case of s.c. fat (Fig. 2*F*). As expected, the adiposity index (sum of the weights of adipose depots expressed as the percentage of total body weight) was also significantly decreased in the HF + IAP group compared with the HF group (Fig. 2*G*), despite the fact that the two groups consumed nearly equal amounts of food (Fig. 2*H*). We noticed that even after just 6 wk of an HFD, mice developed glucose intolerance that was prevented in the IAP-treated group (Fig. S2 *F–I*).

IAP Prevents HFD-Induced Liver Injury. We next examined the efficacy of IAP in preventing HFD-induced liver injury. IAP prevented HFD-induced increase in liver weight (Fig. S3) and also protected mice from HFD-induced increase in the liver enzymes, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and alanine aminotransferase (ALT) (Fig. 3 *A–C*). In addition, compared with the HF mice, the IAP-treated animals had lower levels of total liver lipids (Fig. 3*D*) and triglycerides (TG) (Fig. 3*E*). Finally, histological analyses showed that HF mice had accumulated much higher amounts of hepatic fat, and these changes were not seen in the HF + IAP animals (Fig. 3 *F* and *G*).

IAP Prevents HFD-Induced Dyslipidemia. Lipid profile analyses showed that IAP prevents HFD-induced dyslipidemia (Fig. 4 *A–E*). Most notably, the HDL-C levels markedly increased by ∼73% in the HF + IAP group compared with the HF group (Fig. 4*D*). The HF group receiving IAP supplementation exhibited a great reduction in atherogenic propensity including the important cholesterol ratios and the atherogenic index (Fig. S4 *A–F*).

IAP Prevents HFD-Induced Endotoxemia and Intestinal Permeability. We next sought to assess some of the factors known to underlie metabolic syndrome. Compared with the LF mice, serum endotoxin levels were much higher in the HF group (Fig. 4*F*), whereas these levels were reduced in the HF + IAP group. In addition, compared with the HF group, the endotoxin levels were also greatly reduced in the cecal contents of the IAP-treated group (Fig. S4*G*). To further explore the mechanism by which IAP prevents endotoxemia, we sought to determine whether oral IAP detoxifies LPS within the intestinal lumen or, alternatively, if IAP enters the systemic circulation to detoxify circulating LPS. Accordingly, we treated separate groups of mice with oral or i.p. IAP and oral or i.p. LPS. Oral IAP supplementation blocked the luminal LPS but was unable to prevent endotoxemia induced by two different doses of i.p. LPS (Fig. 4*G* and Fig. S4*H*). In contrast, the i.p. IAP was able to detoxify the i.p. LPS. These results support the concept that oral IAP prevents endotoxemia by detoxifying LPS within the intestinal lumen.

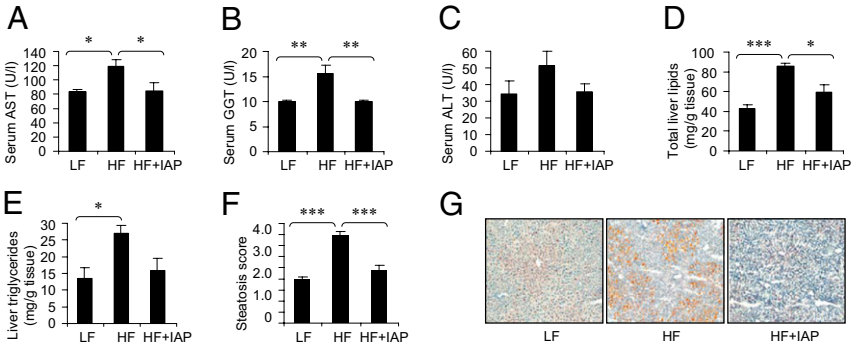

Fig. 3. IAP prevents HFD-induced liver injury. (*A*) Serum aspartate aminotransferase (AST) levels (see Fig. 2 for description of mice). (*B*) Serum gamma-glutamyl transferase (GGT) levels. (*C*) Serum alanine aminotransferase (ALT) levels. (*D*) Total liver lipids. (*E*) Liver triglyceride levels. (*F*) Liver steatosis score. (*G*) Oil Red O staining of frozen liver sections (10× objective). Statistics: data expressed as mean ± SEM. Two-tailed unpaired Student's *t* test. For multiple comparisons, analysis of variance with Tukey was used. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

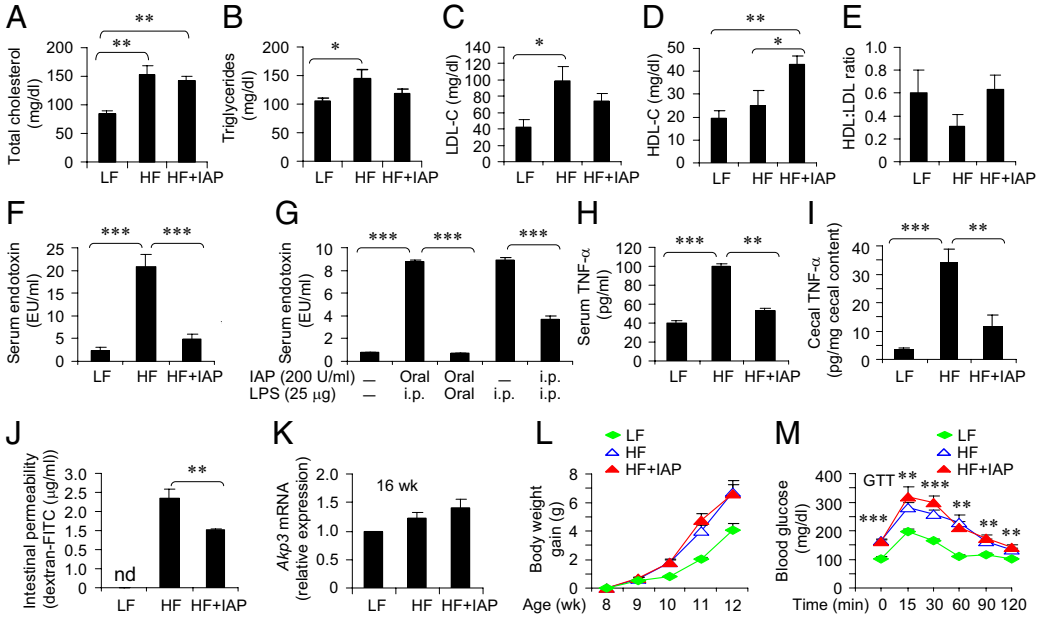

Fig. 4. IAP prevents HFD-induced dyslipidemia and endotoxemia. (*A*) Serum total cholesterol levels (see Fig. 2 for description of mice). (*B*) Levels of serum triglycerides. (*C*) Serum low-density lipoprotein cholesterol (LDL-C) levels. (*D*) Serum high-density lipoprotein cholesterol (HDL-C) levels. (*E*) Ratio between HDL-C and LDL-C. (*F*) Serum endotoxin levels. (*G*) Serum endotoxin levels after i.p. injection of LPS (different groups of mice, see *SI Materials and Methods*). (*H*) Serum tumor necrosis factor-alpha (TNF-α) levels. (*I*) Cecal TNF-α levels. (*J*) Intestinal permeability as determined by quantifying the amount of FITC-dextran (70 kDa) levels in the serum after its oral gavage (different groups of mice, see *SI Materials and Methods*). (*K*) Relative expression of duodenal IAP (*Akp3*) mRNA as determined by qRT-PCR (different groups of mice, see *SI Materials and Methods*). (*L*) Body weight after 4 wk of HFD feeding, which was preceded by 3 wk of oral antibiotics treatment [ampicillin (1 g/L) plus norfloxacin (1 g/L)] (see Fig. S4*P* for energy intake) (different groups of mice, see *SI Materials and Methods*). (*M*) GTT after 4 wk of HFD feeding (see Fig. S4*Q* for AUC) (see *L* for description of mice). Statistics: data expressed as mean ± SEM. Two-tailed unpaired Student's *t* test. For multiple comparisons, analysis of variance with Tukey was used. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. The asterisk (*) refers to the LF vs. HF comparison, and no statistically significant difference was observed between HF and HF + IAP groups (for *M*).

We found that IAP prevented HFD-induced increased levels of serum TNF-α and IL-1β (Fig. 4*H* and Fig. S4*I*, respectively). To further assess the local inflammatory status within the intestine, we measured TNF-α levels in the cecal contents and found approximately threefold higher levels of TNF-α in the HF compared with the HF + IAP mice (Fig. 4*I*). We then directly determined the proinflammatory status of the cecal contents by exposing mouse leukaemic monocyte macrophage RAW264.7 cells to cecal contents and found that the fluid from the HF + IAP mice was less inflammatory (Fig. S4*J*). In addition, we found that the IAP-treated animals were protected from the increase in intestinal permeability that occurs in response to an HFD (Fig. 4*J*). We also studied the acute (10 d) and chronic (16 wk) effects of oral IAP supplementation on the expression of IAP (mouse intestinal alkaline phosphatase gene *Akp3*) mRNA (Fig. 4*K* and Fig. S4*K*), as well as the activity of the endogenous IAP enzyme (Fig. S4 *L* and *M*) and observed no significant differences in IAP expression or activity among the groups.

Based on the known activity of the IAP enzyme and our data on luminal LPS and endotoxemia, it appears that IAP may prevent metabolic syndrome through a mechanism that involves the luminal inhibition of bacterially derived proinflammatory mediators. To further explore this mechanism for IAP action, we performed an experiment using a 3-wk antibiotic regimen to decrease the bacterial content in the gut (Fig. S4 *N* and *O*). We then fed the mice with a high-fat diet ± IAP for 4 wk. Based on measures of body weight (Fig. 4*L* and Fig. S4*P*) and glucose tolerance (Fig. 4*M* and Fig. S4*Q*), it appears that IAP was ineffective in inhibiting the changes of metabolic syndrome in the antibiotic-treated mice, consistent with our hypothesis that IAP may prevent metabolic syndrome by inhibiting ligands derived from luminal bacteria, such as LPS.

IAP Exerts Beneficial Effects in Treating HFD-Associated Metabolic Syndrome. We next investigated whether IAP supplementation would be able to reverse any features of metabolic syndrome. We exposed mice to an HFD for 14 wk to induce metabolic syndrome (Fig. S5 *A–D*) and then treated them with ±calf IAP supplementation (100 units/mL drinking water) for 6 wk. The IAP-treated group showed a significant reduction in glucose intolerance and postglucose hyperinsulinemia (Fig. 5 *A* and *B* and Fig. S5*E*). Fig. 5*C* shows weekly glucose levels during GTT at the 15-min time point, indicating the beneficial effects of IAP. Glucose levels were also significantly reduced in the IAP-treated group during ITT (Fig. 5*D* and Fig. S5*F*). As expected, we observed lower endotoxin levels in the cecal contents (Fig. 5*E*) as well as in serum (Fig. 5*F*) of the HF + IAP group. We found that the HF + IAP group had lower levels of serum TNF-α and IL-1β (Fig. 5 *G* and *H*) and their insulin resistance index was slightly reduced, but not significantly (Fig. S5*G*). During this treatment period, we observed nearly equal energy intake by the HF and HF + IAP mice and IAP treatment did not have any effect on body weight (Fig. S5 *H* and *I*). We saw slight improvement in the dyslipidemia and extent of liver injury, but in this short experiment these differences did not reach statistical significance.

Low-Fat Diet Supplemented with Oral IAP Improves Lipid Profile. We next sought to determine whether oral supplementation with IAP would have any beneficial effects in mice exposed to an LFD. We fed 5-wk-old female mice a LFD ± IAP (100 units/mL drinking water) for 7 wk. Mice receiving the LFD + IAP appeared to show slight improvement in glucose tolerance and insulin sensitivity, but these differences were not significant. On the other hand, we found dramatic beneficial effects in the case of the lipid profile (Fig. 6 *A–E*). The IAP-treated mice had higher levels of HDL-C (Fig. 6*D*) and greatly increased HDL-C:

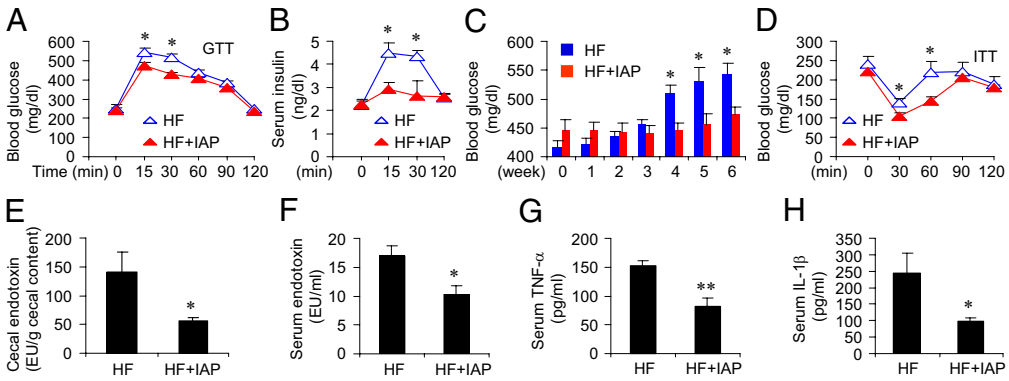

Fig. 5. IAP cures HFD-induced metabolic syndrome. Groups of 5-wk-old WT male C57BL/6 mice ($n = 5$ for each group) were fed HFD (45% kcal from fat) for 14 wk to induce metabolic syndrome (Fig. S5). Mice with metabolic syndrome were then treated with or without IAP (100 units/mL drinking water) for 6 wk. (*A*) Blood glucose levels during GTT. (*B*) Serum insulin levels during GTT. (*C*) Weekly blood glucose levels during GTT at 15-min time point. (*D*) Blood glucose levels during ITT. (*E*) Cecal endotoxin levels. (*F*) Serum endotoxin levels. (*G*) Serum TNF-α levels. (*H*) Serum IL-1β levels. Statistics: data expressed as mean ± SEM. Two-tailed unpaired Student's *t* test. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

LDL-C ratio (Fig. 6*E*). We also calculated the various atherogenic indices and found that the LF + IAP group had a great reduction in the atherogenesis risk (Fig. S6 *A*–*F*).

Discussion

Metabolic syndrome is one of the most important modern global health problems. The etiology of HFD-induced metabolic syndrome is thought to be related to metabolic endotoxemia (19). An HFD has also been associated with an imbalance in the normal composition and number of microbes in the gut (dysbiosis), resulting in barrier dysfunction followed by LPS translocation to the systemic circulation (20).

The present study was undertaken based upon work in our laboratory and others that points to the role for IAP in protecting the host from bacterial toxins (16). The present data in mice provide a "proof of principle" that IAP could be an effective oral supplement against endotoxemia, thus protecting the host from metabolic syndrome. We have shown that IAP reduces corn-oil–induced endotoxemia and prevents the inflammation and intestinal permeability changes that occur in response to an HFD. Interestingly, the excess secretion of IAP seen in rats fed an HFD (21) could represent a physiological response of the body to protect against HFD-associated systemic inflammation. IAP is primarily expressed in the enterocytes of the proximal small intestine and is bidirectionally secreted into the intestinal lumen as well as the systemic circulation (22). IAP is strictly conserved among species (23) and exists within a microbial environment that highlights its role in the detoxification of bacterial proinflammatory factors as well as in the prevention of dysbiosis (16, 24). Various isozymes of alkaline phosphatases (APs) exist, namely IAP, placental AP, tissue nonspecific (liver/bone/kidney/neutrophils) AP, and germ cell AP (23). These various AP enzymes share significant structural homology as well as functional similarities. It will be interesting in future studies to determine whether these other AP isozymes also have the capability to protect the host from metabolic syndrome.

IAP exists within an environment that contains a wide range of bacterially derived proinflammatory factors and we have previously demonstrated that in addition to LPS, other targets for the IAP enzyme include flagellin and CpG DNA (16). In regard to metabolic syndrome, although LPS has been the most intensively studied gut-derived inflammatory mediator, it is likely that it is not the only one because the other mediators also activate the NF-κB pathway and ultimately increase the levels of proinflammatory cytokines (25). As such, we speculate that the beneficial effects of IAP may be due to its detoxification of a number of proinflammatory factors, not just LPS. Our results using antibiotics to decrease the luminal bacterial content before the HFD ± IAP add support to the idea that the mechanism of IAP action largely involves its ability to block luminal, bacterially derived inflammatory mediators. Of course, we are unable to completely rule out the possibility that IAP may also be exerting some impact on metabolic syndrome through a mechanism that is independent of luminal bacterial products.

In regard to the gut microflora, we have previously reported that IAP-KO mice display an overall decrease in the number of intestinal bacteria and oral supplementation with IAP in WT mice was able to rapidly restore the normal gut flora in mice exposed to antibiotics (24). The prevention of HFD-induced metabolic endotoxemia in this study could be due in part to the role of IAP in preventing HFD-induced gut dysbiosis, although our acute experiments with corn oil and LPS show an inhibitory impact of IAP that is independent of any chronic changes in the flora. The critical role for IAP at the interface between the host and the intestinal lumen is supported by studies in zebrafish (26), who found that IAP expression was induced only when the fish were exposed to bacteria, whereas the enzyme was absent under germ-free conditions. Interestingly, Jang et al. (27) showed that

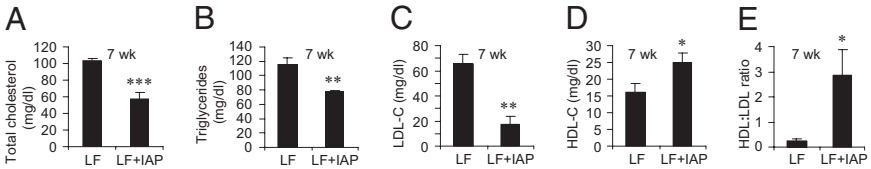

Fig. 6. Low-fat diet (LFD) supplemented with IAP improves lipid profile. Groups of 5-wk-old WT female C57BL/6 mice ($n = 5$) were fed LFD (14% kcal from fat) ± IAP (100 units/mL drinking water) for 7 wk. (*A*) Serum total cholesterol levels. (*B*) Serum triglyceride levels. (*C*) Serum LDL-C levels. (*D*) Serum HDL-C levels. (*E*) Ratio between HDL-C and LDL-C. Statistics: data expressed as mean ± SEM. Two-tailed unpaired Student's *t* test. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

endogenous IAP levels in rats decrease with age, indicating a possible role of "loss of IAP" as a precipitating cause of metabolic syndrome that is known to be common with aging (28).

In a therapeutic context, Lukas et al. (29) reported on a single arm study in humans with severe ulcerative colitis and showed that enterally administered bovine IAP is extremely safe. We have previously shown that oral IAP supplementation in mice increases IAP concentrations in stools in a dose-dependent manner (24), suggesting that oral IAP dose could be easily adjusted to achieve an effective therapeutic level.

In summary, we have demonstrated that oral IAP supplementation both prevents an HFD-induced metabolic syndrome and reverses the changes associated with an HFD-induced metabolic syndrome. Furthermore, IAP can also improve the lipid profile during an LFD feeding. Although caution needs to be taken in extrapolating the findings in mouse models of inflammation to human diseases (30), taken together, the present findings suggest that oral IAP supplementation in humans could represent a safe, effective, and unique approach to the prevention or treatment of metabolic syndrome.

Materials and Methods

C57BL/6 IAP-KO (*Akp3*$^{-/-}$) mice were generated by the Sanford-Burnham Medical Research Institute (La Jolla, CA) and have been described (18). IAP-KO and WT littermates were bred at the Massachusetts General Hospital (MGH) animal facility. All animal work protocols were reviewed and approved by the Institutional Animal Care and Use Committee at MGH. Groups of age- and sex-matched WT and IAP-KO mice ($n = 5$) were fed an LFD (14% kcal from fat) or HFD (45% kcal from fat) for the specified periods of time (see *SI Materials and Methods* for details) with or without calf IAP (100 units/mL drinking water). Established protocols were followed to determine insulin resistance (5), dyslipidemia (18), endotoxemia (5), and hepatic enzyme levels (15). Frozen liver tissues were submitted to the histology laboratory at MGH to prepare Oil Red O stained slides. Details of methods are described in *SI Materials and Methods*.

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1. Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr., Lenfant C; American Heart Association; National Heart, Lung, and Blood Institute (2004) Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 109(3): 433–438.
2. Hotamisligil GS (2006) Inflammation and metabolic disorders. *Nature* 444(7121): 860–867.
3. Ford ES (2005) Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the U.S. *Diabetes Care* 28(11):2745–2749.
4. Malik S, et al. (2004) Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. *Circulation* 110(10):1245–1250.
5. Cani PD, et al. (2007) Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 56(7):1761–1772.
6. Hohmeier HE, Tran VV, Chen G, Gasa R, Newgard CB (2003) Inflammatory mechanisms in diabetes: Lessons from the beta-cell. *Int J Obes Relat Metab Disord* 27(Suppl 3): S12–S16.
7. Gieling RG, Wallace K, Han YP (2009) Interleukin-1 participates in the progression from liver injury to fibrosis. *Am J Physiol Gastrointest Liver Physiol* 296(6):G1324–G1331.
8. Hansson GK (2005) Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 352(16):1685–1695.
9. Pendyala S, Walker JM, Holt PR (2012) A high-fat diet is associated with endotoxemia that originates from the gut. *Gastroenterology* 142:1100–1101 e1102.
10. Ghoshal S, Witta J, Zhong J, de Villiers W, Eckhardt E (2009) Chylomicrons promote intestinal absorption of lipopolysaccharides. *J Lipid Res* 50(1):90–97.
11. Ding S, et al. (2010) High-fat diet: Bacteria interactions promote intestinal inflammation which precedes and correlates with obesity and insulin resistance in mouse. *PLoS ONE* 5(8):e12191.
12. Park EJ, et al. (2007) Dietary ganglioside inhibits acute inflammatory signals in intestinal mucosa and blood induced by systemic inflammation of Escherichia coli lipopolysaccharide. *Shock* 28(1):112–117.
13. Bruewer M, et al. (2003) Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. *J Immunol* 171(11):6164–6172.
14. Cani PD, et al. (2008) Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 57(6):1470–1481.
15. Tsukumo DM, et al. (2007) Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. *Diabetes* 56(8):1986–1998.
16. Chen KT, et al. (2010) Identification of specific targets for the gut mucosal defense factor intestinal alkaline phosphatase. *Am J Physiol Gastrointest Liver Physiol* 299(2): G467–G475.
17. Koyama I, Matsunaga T, Harada T, Hokari S, Komoda T (2002) Alkaline phosphatases reduce toxicity of lipopolysaccharides in vivo and in vitro through dephosphorylation. *Clin Biochem* 35(6):455–461.
18. Narisawa S, et al. (2003) Accelerated fat absorption in intestinal alkaline phosphatase knockout mice. *Mol Cell Biol* 23(21):7525–7530.
19. Moreira AP, Texeira TF, Ferreira AB, Peluzio MdoC, Alfenas RdeC (2012) Influence of a high-fat diet on gut microbiota, intestinal permeability and metabolic endotoxaemia. *Br J Nutr* 108(5):801–809.
20. de La Serre CB, et al. (2010) Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. *Am J Physiol Gastrointest Liver Physiol* 299(2):G440–G448.
21. Mahmood A, Shao JS, Alpers DH (2003) Rat enterocytes secrete SLPs containing alkaline phosphatase and cubilin in response to corn oil feeding. *Am J Physiol Gastrointest Liver Physiol* 285(2):G433–G441.
22. Eliakim R, Mahmood A, Alpers DH (1991) Rat intestinal alkaline phosphatase secretion into lumen and serum is coordinately regulated. *Biochim Biophys Acta* 1091(1): 1–8.
23. Goldstein DJ, Rogers CE, Harris H (1980) Expression of alkaline phosphatase loci in mammalian tissues. *Proc Natl Acad Sci USA* 77(5):2857–2860.
24. Malo MS, et al. (2010) Intestinal alkaline phosphatase preserves the normal homeostasis of gut microbiota. *Gut* 59(11):1476–1484.
25. Kaisho T, Akira S (2006) Toll-like receptor function and signaling. *J Allergy Clin Immunol* 117(5):979–987, quiz 988.
26. Bates JM, Akerlund J, Mittge E, Guillemin K (2007) Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota. *Cell Host Microbe* 2(6):371–382.
27. Jang I, Jung K, Cho J (2000) Influence of age on duodenal brush border membrane and specific activities of brush border membrane enzymes in Wistar rats. *Exp Anim* 49(4):281–287.
28. Bechtold M, Palmer J, Valtos J, Iasiello C, Sowers J (2006) Metabolic syndrome in the elderly. *Curr Diab Rep* 6(1):64–71.
29. Lukas M, et al. (2010) Exogenous alkaline phosphatase for the treatment of patients with moderate to severe ulcerative colitis. *Inflamm Bowel Dis* 16(7):1180–1186.
30. Seok J, et al.; Inflammation and Host Response to Injury, Large Scale Collaborative Research Program (2013) Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci USA* 110(9):3507–3512.