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# *Porphyromonas gingivalis* Induces Insulin Resistance by Increasing BCAA Levels in Mice

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**Abstract**

Insulin resistance is one of the critical pathogeneses of type 2 diabetes mellitus (T2DM). Elevated levels of plasma branched-chain amino acids (BCAAs) are associated with insulin resistance. Recent studies have demonstrated the role of *Porphyromonas gingivalis* in the development of insulin resistance. However, the mechanisms by which *P. gingivalis* induces insulin resistance are still unclear. The purpose of this study was to investigate whether *P. gingivalis* induces insulin resistance through BCAA biosynthesis. We established a murine model of periodontitis by infecting mice with *P. gingivalis*. Alveolar bone loss, insulin sensitivity, and the plasma level of BCAAs were measured. A *P. gingivalis* BCAA aminotransferase-deficient strain (*Δbcat*) was constructed, and its kinetic growth, biofilm formation, and in vivo colonization were compared with its wild-type strain. Alveolar bone loss, insulin sensitivity, and the plasma level of BCAAs of the mice infected with either wild-type strain or *Δbcat* strain were further measured. We found that periodontal infection with *P. gingivalis* significantly upregulated the plasma level of BCAAs and aggravated the high-fat diet (HFD)–induced insulin resistance. *Bcat* deletion did not alter the growth, biofilm formation, and in vivo colonization of *P. gingivalis*. More important, the *Δbcat* strain was unable to upregulate the plasma level of BCAAs and induce insulin resistance in HFD-fed mice. These findings suggest that the BCAA biosynthesis of *P. gingivalis* plays a critical role in the development of insulin resistance in the HFD-fed mice. The BCAA biosynthesis pathways may provide a potential target for the disruption of linkage between periodontitis and T2DM.

**Keywords:** branched-chain amino acids, periodontitis, diabetes mellitus, high-fat diet, alveolar bone loss, blood glucose

## Introduction

Periodontitis is induced by the dysbiosis of oral microbial communities and characterized by inflammatory destruction of periodontal tissues that support the tooth. The red complex bacteria, including *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola*, are closely related to the development of periodontitis (Tokutomi et al. 2015). *P. gingivalis* has been strongly implicated as a pathogen in chronic periodontitis (Lamont et al. 2018). The detection rate of *P. gingivalis* in the periodontitis population is 79% to 90%, and that in the healthy population is 10% to 25% (Igboin et al. 2009). *P. gingivalis* has a specific arsenal of virulence factors that enable its invasion to the periodontal tissue and subsequent dissemination into the systemic circulation, increasing the risk of systemic chronic diseases such as type 2 diabetes mellitus (T2DM), cardiovascular diseases, nonalcoholic fatty liver disease (NAFLD), rheumatoid arthritis, and Alzheimer disease (Maekawa et al. 2011; Olsen et al. 2016; Olsen and Yilmaz 2016; Potempa et al. 2017; Singhrao and Olsen 2019).

Insulin resistance refers to the reduction or loss of the response of the target organs and tissues to the biological effects of insulin, resulting in decreased efficiency of cell uptake and utilization of glucose and the occurrence of abnormal metabolism of glucose and lipids in cells. Insulin resistance is well recognized as the main pathological feature of T2DM (Czech 2017). *P. gingivalis* has been suggested to be involved in the occurrence and development of insulin resistance (Makiura et al. 2008; Aemaimanan et al. 2013; Arimatsu et al. 2014). Repeated oral administration of *P. gingivalis* elicited endotoxemia and induced insulin resistance in mice (Arimatsu et al. 2014). The level of HbA1c in patients with T2DM was positively correlated with the number of the red complex bacteria in the periodontal pocket (Aemaimanan et al.

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A supplemental appendix to this article is available online.

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2013), suggesting that poor blood glucose control in patients with diabetes might be related to the increased periodontal load of red complex bacteria. A longitudinal study on the subgingival plaque of patients with periodontitis and T2DM showed that glycemic levels in diabetes were affected by the persistence of *P. gingivalis* in periodontal pockets, especially clones with type II fimbriae (Makiura et al. 2008). Nonsurgical periodontal therapy not only alleviated periodontal inflammation but also improved glucose metabolism and insulin sensitivity in patients with T2DM (Navarro-Sanchez et al. 2007).

Branched-chain amino acids (BCAAs), including leucine, isoleucine, and valine, are essential amino acids mainly biosynthesized by fungi, plants, and bacteria. In the late 1960s, an elevated plasma level of BCAAs in patients with impaired insulin signaling was first described (Felig et al. 1969). Recently, many prospective and cross-sectional human studies have consistently demonstrated that an increased plasma level of BCAAs was associated with obesity, insulin resistance, and diabetes (Newgard et al. 2009; Menni et al. 2013; Nakamura et al. 2014). BCAAs also have been considered predictors for the development of T2DM (Yamakado et al. 2015). The role of BCAAs in the development of insulin resistance and T2DM was evidenced by multiple studies that proposed a persistent activation of the mammalian target of rapamycin (mTOR) (Newgard et al. 2009; Xiao et al. 2014). BCAAs can activate mTOR and subsequently activate ribosomal protein S6 kinase1 (S6K1), which phosphorylates insulin receptor substrate 1 (IRS-1), resulting in insulin resistance (Yoon 2016).

Recently, gut bacteria *Prevotella copri* and *Bacteroides vulgatus* have been implicated as the major contributors that link the microbial biosynthesis of BCAAs and insulin resistance. *P. copri* can aggravate glucose intolerance and augment the circulating level of BCAAs and consequently induce insulin resistance in mice (Pedersen et al. 2016). Intriguingly, *P. gingivalis* also possesses a BCAA biosynthetic pathway where branched-chain amino acid aminotransferase (*bcat*) encoded by *PGN_RS05180* (gene ID: 29256289) functions as a key enzyme. Hence, we hypothesized that *P. gingivalis* may induce insulin resistance through BCAA biosynthesis. Specifically, we established a *P. gingivalis*–induced periodontitis mouse model and demonstrated that *P. gingivalis* could aggravate insulin resistance in the high-fat diet (HFD)–fed mice, and this effect was dependent on its BCAA biosynthesis.

## Materials and Methods

### Bacteria and Culture Conditions

*P. gingivalis* ATCC 33277 was cultured on 5% sheep blood agar supplemented with 5 mg/L hemin and 0.5 mg/L menadione at 37°C and incubated under anaerobic conditions (80% $N_2$, 10% $CO_2$, and 10% $H_2$). The *P. gingivalis* branched-chain amino acid aminotransferase deficient strain (Δ*bcat*) was constructed by homologous recombination (Appendix Fig. 1). The primers used are shown in Appendix Table 1. The DNA sequencing result confirmed the success of mutant construction (Appendix Fig. 2).

### Animals and Study Design

All animal procedures in this study were conducted in strict accordance with the guidelines of the Ethics Committee of West China Hospital of Stomatology, Sichuan University, and an ethics approval was obtained (license number WCHSIRB-D-2016-192). Five-week-old male C57BL/6 mice (Da Shuo) were housed in specific pathogen-free facilities.

As shown in Appendix Figure 3, in experiment 1, after 1 wk of environment acclimation, the mice were randomly divided into 4 groups using a random-number table ($n = 6$ per group): *P. gingivalis* + high-fat diet (*Pg* + HFD), *P. gingivalis* + normal chow (*Pg* + NC), vehicle + high-fat diet (HFD), vehicle + normal chow (NC). In experiment 2, after 1 wk of environment acclimation, the mice were randomly divided into 3 groups ($n = 6$ per group): *P. gingivalis* wild type + high-fat diet (wild type + HFD), Δ*bcat* + high-fat diet (Δ*bcat* + HFD), and vehicle + high-fat diet (HFD).

HFD-fed mice were fed with a high-fat diet (60% fat, Research Diets No. D12492; Research Diets) for 12 wk, whereas NC-fed mice were fed with a normal chow (10% fat, Research Diets No. D12450J). Feed formula of D12492 and D12450J is shown in Appendix Table 2.

The periodontitis mouse model was established according to the protocol described by Li and Amar (2007) with slight modifications. All mice were ligated with a 5-0 silk suture around the right maxillary first molars for experimental periodontitis. The overnight bacterial cultures (wild type/Δ*bcat*) were suspended in phosphate-buffered saline (PBS) with 2% carboxymethyl cellulose (Tokyo Chemical Industry) at a defined microbial population of $1 \times 10^9$ colony-forming units (CFU)/mL. Small sterile cotton soaked with 100 μL of bacterial resuspension was applied to the ligated teeth of the mice (anesthetized with ether) twice per week for 4 wk. HFD and NC groups were sham-administered with *P. gingivalis*–free PBS with 2% carboxymethyl cellulose as controls.

### Glucose Tolerance, Fasting Blood Glucose, Fasting Insulin, and Insulin Sensitivity

An oral glucose tolerance test (OGTT) was performed at the end of the 13th week to assess the glucose tolerance. The mice were fasted for 6 h with free access to water and gavaged with 2 g glucose/kg (Andrikopoulos et al. 2008). Blood glucose was measured in tail vein blood before (fasting blood glucose) and 15, 30, 60, 90, and 120 min after glucose administration, using a glucometer (Roche). The OGTT curve was drawn and the area under the curve (AUC) was calculated. AUC = a/2 + b + c/2 (a, b, c represented blood glucose values before and 60 and 120 min after glucose administration). The unit for blood glucose was mmol/L (Zhang et al. 2015).

The mice were fasted for 6 h before blood for insulin measurement was collected in heparin sodium–coated tubes kept on ice. The blood was centrifuged at 4°C for 10 min at 1,000 *g*, and plasma was then collected and stored at −80°C until use. The level of plasma insulin was measured with enzyme-linked immunosorbent assay (ELISA) kits (Mercodia) according to

the manufacturer's protocol. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated to assess the insulin sensitivity. HOMA-IR = fasting blood glucose (mmol/L) × fasting insulin (mIU) / 22.5 (Zhang et al. 2015).

### *Plasma Level of BCAAs*

Blood was collected by retro-orbital bleeding of the mice 3 d after OGTT and insulin measurement with heparin sodium–coated tubes on ice. The blood was centrifuged at 4°C for 10 min at 1,000 *g*, and the plasma was then collected and stored at −80°C until metabolomic analysis. The level of plasma BCAAs was detected by liquid chromatography–mass spectrometry (LC-MS).

### *Alveolar Bone Loss*

The mice were sacrificed immediately after blood collection for BCAA measurement by cervical dislocation, and the right maxillae were dissected free of soft tissues to evaluate alveolar bone loss. The maxillae were scanned using micro–computed tomography (μCT50; SCANCO). Data were acquired at 70 kVp, with a 10-μm isotropic voxel size. The alveolar bone loss (mm) was measured from the cementoenamel junction (CEJ) to the alveolar bone crest (ABC) of the first molar.

### *Kinetic Growth of* P. gingivalis

The overnight culture of *P. gingivalis* was diluted to an optical density ($OD_{600nm}$) of 0.01 and then anaerobically cultured in Brain Heart Infusion supplemented with 5 mg/L hemin and 0.5 mg/L menadione (BHI) at 37°C. Then, 1 mL of the bacterial culture was taken into cuvettes at 4, 8, 12, 16, 20, 24, 32, 36, 40, 44, and 48 h, and the $OD_{600nm}$ values were measured using a microplate reader (Multiskan GO; Thermo Scientific) after shaking and mixing. The experiment was repeated 3 times, and the growth curves were depicted.

### *Biofilm Formation*

Biofilm formation of *P. gingivalis* was examined by crystal violet staining. Then, 2 mL of overnight culture of *P. gingivalis* was grown in BHI for 96 h at 37°C in a 24-well plate. The culture medium was changed every 24 h. The supernatant was removed and the adherent biofilms were rinsed for 3 times using PBS. The adherent biofilms were then incubated with methanol for 15 min followed by staining with 0.1% crystal violet for 5 min. After washing with PBS, the plate was dried overnight. Finally, 200 μL of 30% acetic acid was used to dissolve the stain, and the plate was shaken for 30 min at room temperature before the eluate was transferred to another new plate. The $OD_{600nm}$ values of the eluate were recorded to reflect the biomass of biofilm.

### *Quantification of Salivary* P. gingivalis

To evaluate the colonization of *P. gingivalis* in the oral cavity, the salivary carriage of *P. gingivalis* at the 13th week was analyzed by quantitative polymerase chain reaction (qPCR). Total DNA extraction was performed using a DNA extraction kit (QIAGEN) according to the manufacturer's protocol. Isolated DNA was then suspended in sterile DNA-free water and quantified using a spectrophotometer.

A standard curve (Appendix Fig. 4) was constructed by cycle threshold ($C_T$) values of 10-fold serial dilution of the DNA products purified after PCR amplification (template: *P. gingivalis* DNA; primer: *P. gingivalis* forward 5′-AGGCAGC TTGCCATACTGCG-3′ and *P. gingivalis* reverse 5′-ACTG TTAGCAACTACCGATGT-3′) and its copies. The copies of PCR products were calculated by the following formula (Lee et al. 2006): DNA (copies/μL) = $6.02 \times 10^{23}$ (copies/mol) × DNA concentration (ng/μL) × $10^{-9}$ (g/ng) /DNA length (bp) /660 (g/mol/bp).

DNA samples were amplified using appropriate primers (*P. gingivalis* forward 5′- AGGCAGCTTGCCATACTGCG-3′ and *P. gingivalis* reverse 5′- ACTGTTAGCAACTACCGAT GT-3′) and qPCR reagent (SYBR Premix Ex Taq II; Takara) in a real-time PCR equipment (Roche). Amplification reactions were conducted as follows: initial denaturation at 95°C for 30 s, denaturation at 95°C for 5 s, annealing/extension at 60°C for 30 s, and 40 cycles for amplification. The corresponding copy number was calculated using the standard curve.

### *Statistical Analyses*

All data were statistically analyzed by SPSS 21.0 statistical software (SPSS, Inc.). The measurement data were expressed as mean ± standard deviation (mean ± SD). The normality of the distribution of each variable was assessed by means of the Shapiro-Wilk test, and then Levene's test was used to verify the homogeneity of variances. Differences between groups were evaluated by 1-way analysis of variance (ANOVA) with Tukey for multiple comparisons, except for qPCR data that were analyzed by the Games-Howell test. A 2-tailed $P < 0.05$ was considered significant.

## Results

### P. gingivalis *Aggravates HFD-Induced Insulin Resistance*

No significant difference in the body weight of HFD-fed mice and the corresponding NC-fed mice was observed at either baseline or the end of experiment (Appendix Table 3). *P. gingivalis* induced significantly increased alveolar bone loss compared to the noninfected group, confirming the establishment of periodontitis (Fig. 1A, B). We further measured the glucose metabolism in response to nutritional stress to explore the effect of *P. gingivalis* and high-fat diet on insulin sensitivity. OGTT showed that the glucose tolerance was reduced in the HFD group when compared to the NC group, and that was further decreased in the *Pg* + HFD group, suggesting that the HFD-induced glucose intolerance can be further aggravated by periodontal infection of *P. gingivalis* (Fig. 1C). Notably, no

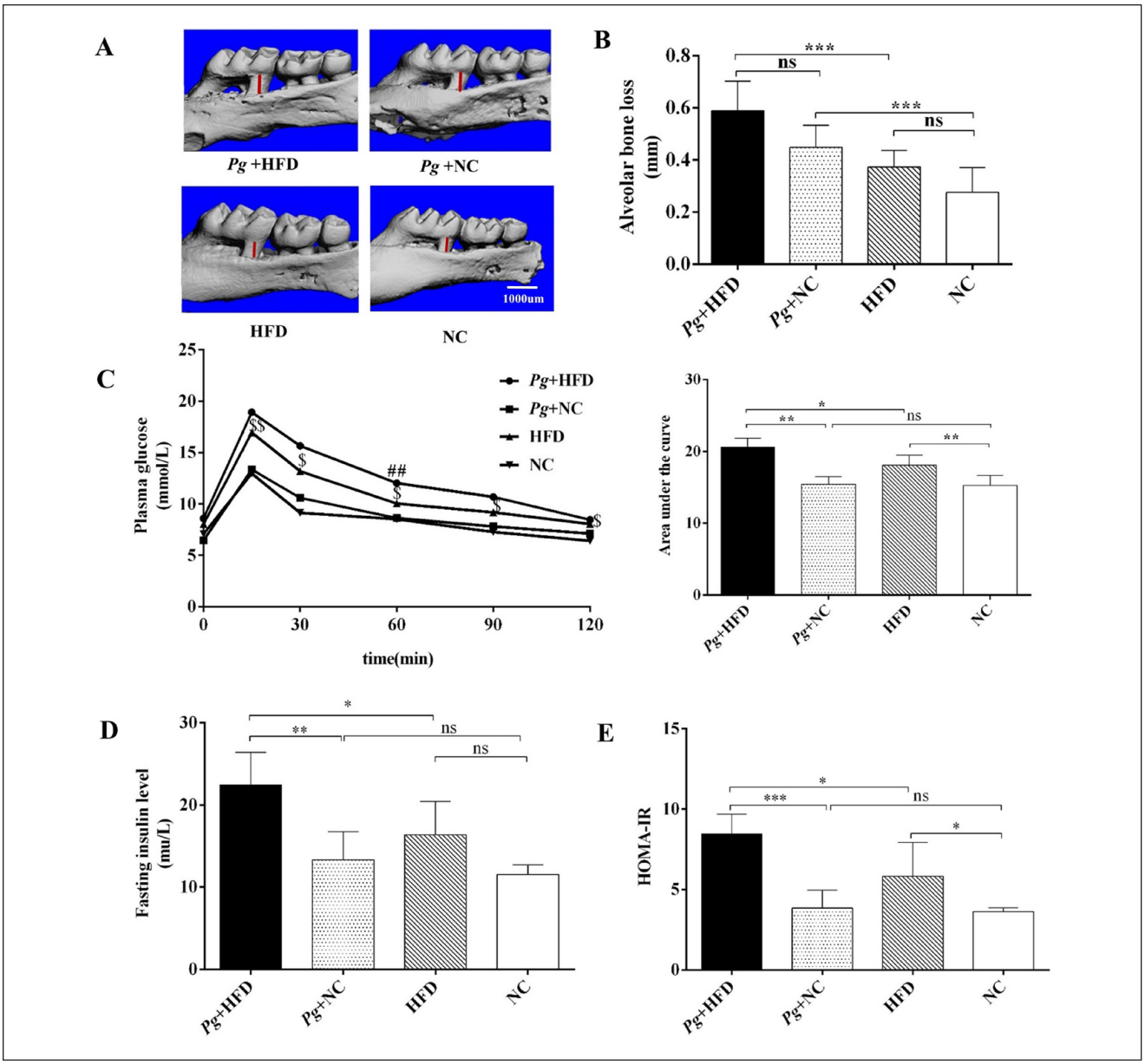


**Figure 1.** Effects of periodontal infection of *Porphyromonas gingivalis* on the alveolar bone loss, glucose tolerance, and insulin sensitivity. (**A**) Micro–computed tomography (CT) reconstruction showing alveolar bone loss at the interproximal sites (red line) of maxillary first molars (buccal view). (**B**) Quantitative analyses of alveolar bone loss by micro-CT. (**C**) Glucose tolerance as reflected by oral glucose tolerance test and area under the curve at the 13th week. (**D**) Fasting insulin levels of the mice at the 13th week. (**E**) Homeostasis model assessment of insulin resistance (HOMA-IR) calculated by measuring fasting blood glucose and fasting insulin levels in mice. $N = 6$ mice per group. Data are presented as mean ± SD. $^{\#\#}P < 0.01$ when compared to high-fat diet (HFD). $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ when compared to normal chow (NC). $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.005$. ns, not significant.

significant differences in blood glucose levels and AUC were observed between NC and *Pg* + NC groups (Fig. 1C).

We measured the level of fasting insulin and calculated HOMA-IR to further evaluate insulin sensitivity. A higher HOMA-IR value reflects a lower insulin sensitivity. The level of fasting insulin in the *Pg* + HFD group was higher than that in the HFD group (Fig. 1D). HOMA-IR in the HFD group was higher than that in the NC group, while that in the *Pg* + HFD group was significantly higher than the HFD group (Fig. 1E). However, HOMA-IR in the *Pg* + NC and NC groups was not statistically different (Fig. 1E). All these data showed that *P. gingivalis* aggravated the HFD-induced insulin resistance, but it had no effect on insulin sensitivity of the NC-fed mice.

## P. gingivalis *Increases Plasma Level of BCAAs in Mice*

The plasma levels of leucine, isoleucine, valine, and total BCAAs of mice infected by *P. gingivalis* were significantly higher than that in the noninfected groups, regardless of the

type of diet. No significant differences in leucine, isoleucine, valine and total BCAA levels were observed between the HFD- and NC-fed mice (Fig. 2A–D), suggesting that periodontal infection with *P. gingivalis* can elevate plasma level of BCAAs that may aggravate insulin resistance.

## Δbcat *Strain Loses the Ability to Increase Plasma Level of BCAAs in Mice*

The in vitro growth and biofilm formation of the branched-chain amino acid aminotransferase deficient strain (Δ*bcat*) was evaluated. The Δ*bcat* strain showed comparable growth and biofilm formation to its wild-type strain (Fig. 3A, B). The in vivo colonization of the *Δbcat* strain in the saliva of mice was further quantified by qPCR. No significant difference in the salivary load of *P. gingivalis* and its *Δbcat* strain was observed (Fig. 3C), suggesting a comparable in vivo colonization of the *Δbcat* and its wild-type strain. In addition, no significant difference in alveolar bone loss was observed between the wild-type + HFD and *Δbcat* + HFD groups (Fig. 3D), indicating that *bcat* deletion did not significantly reduce the periodontal virulence of *P. gingivalis*.

The plasma levels of leucine, isoleucine, valine, and total BCAAs were significantly higher in the wild-type + HFD group than in the other 2 groups, and no significant difference regarding the level of BCAAs was observed between *Δbcat* + HFD and HFD groups (Fig. 4A–D).

## Δbcat *Strain Is Not Able to Induce Insulin Resistance in HFD-Mice*

AUC of the wild-type + HFD group was greater than that of the other 2 groups. No difference in AUC was observed between the *Δbcat* + HFD and HFD groups (Fig. 5A). The level of fasting insulin of the wild-type + HFD group was higher than that of the *Δbcat* + HFD group (Fig. 5B). Importantly, HOMA-IR was significantly higher in the wild-type + HFD group than in the other 2 groups, and no significant difference was observed between the *Δbcat* + HFD and HFD groups (Fig. 5C).

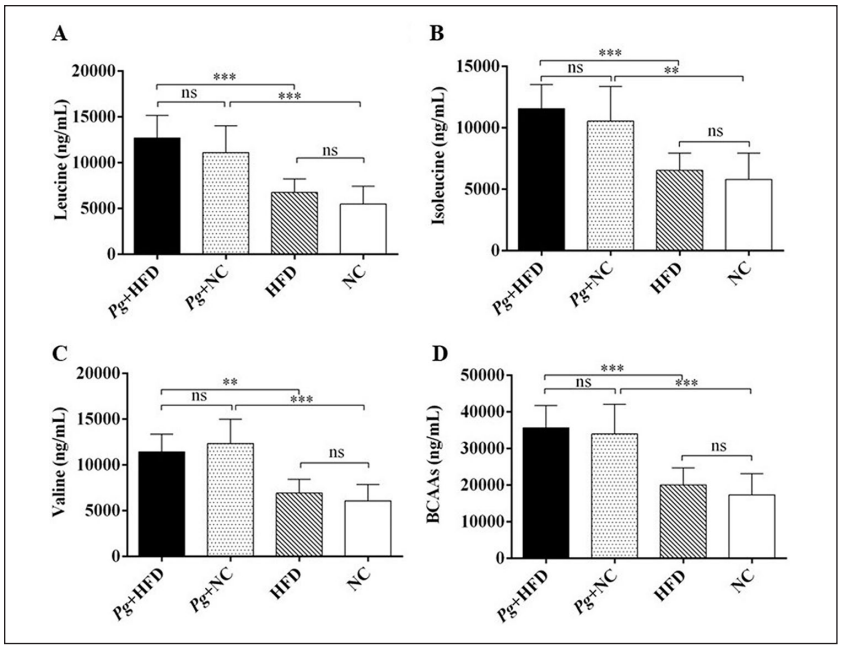


**Figure 2.** Periodontal infection of *Porphyromonas gingivalis* increases plasma level of branched-chain amino acids (BCAAs) in mice. Plasma level of leucine (**A**), isoleucine (**B**), valine (**C**), and total BCAAs (**D**) as determined by liquid chromatography–mass spectrometry before sacrifice. $N = 6$ mice per group. Data are presented as mean ± SD. HFD, high-fat diet; NC, normal chow. $^{**}P < 0.01$. $^{***}P < 0.005$. ns, not significant.

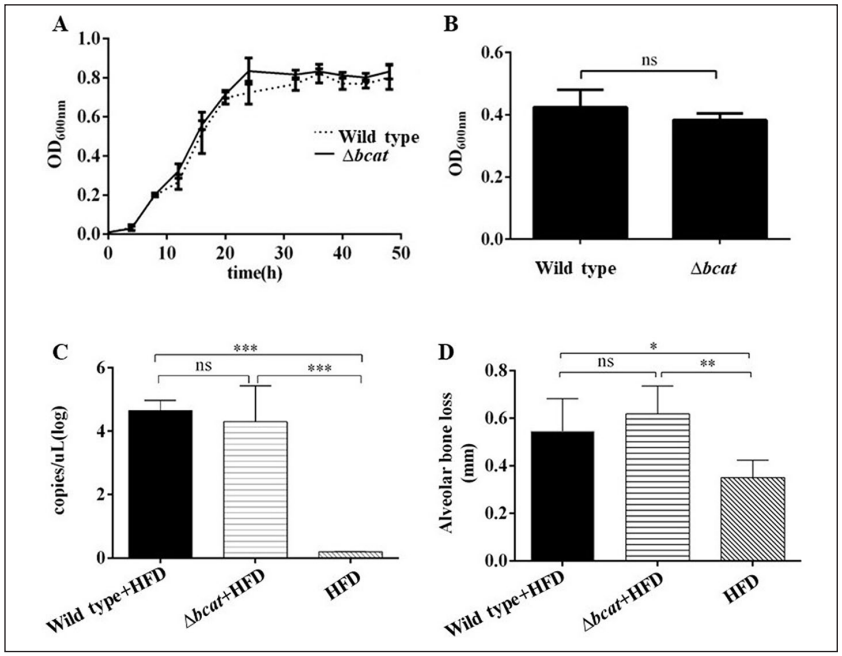


**Figure 3.** *Porphyromonas gingivalis* and its Δ*bcat* strain showed comparable kinetic growth, biofilm formation, in vivo colonization, and periodontal pathogenicity. (**A**) Growth curve of *P. gingivalis* wild type and *Δbcat*. (**B**) Biofilm formation of *P. gingivalis* wild type and *Δbcat*. (**C**) Salivary carriage of *P. gingivals* in mice as quantified by quantitative polymerase chain reaction. (**D**) Quantitative analyses of alveolar bone loss by micro–computed tomography. $N = 6$ mice per group. Data are presented as mean ± SD. HFD, high-fat diet; NC, normal chow. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.005$. ns, not significant.

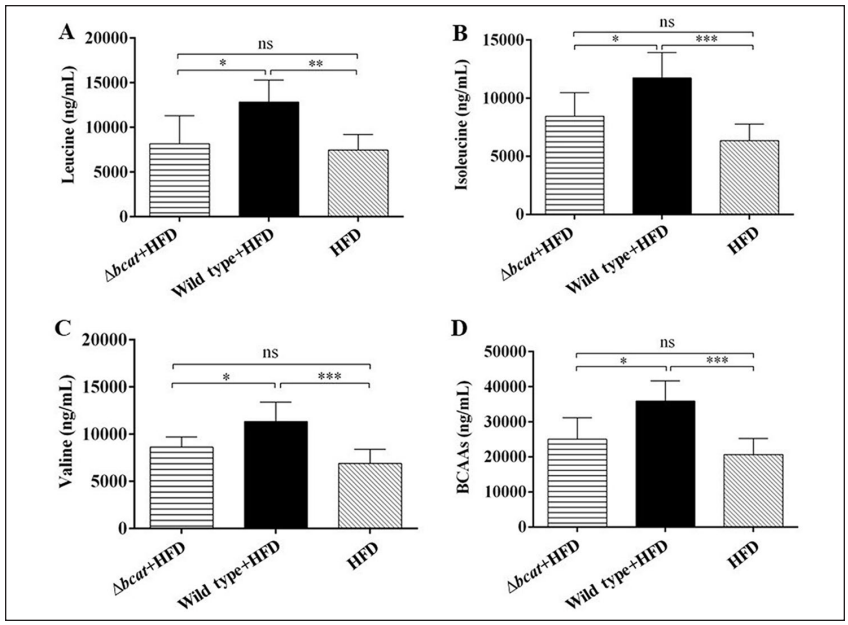


**Figure 4.** Δ*bcat* strain loses the ability to increase plasma branched-chain amino acid (BCAA) levels in mice. Plasma level of leucine (**A**), isoleucine (**B**), valine (**C**), and total BCAAs (**D**) as determined by liquid chromatography–mass spectrometry. $N = 6$ mice per group. Data are presented as mean ± SD. HFD, high-fat diet. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.005$. ns, not significant.

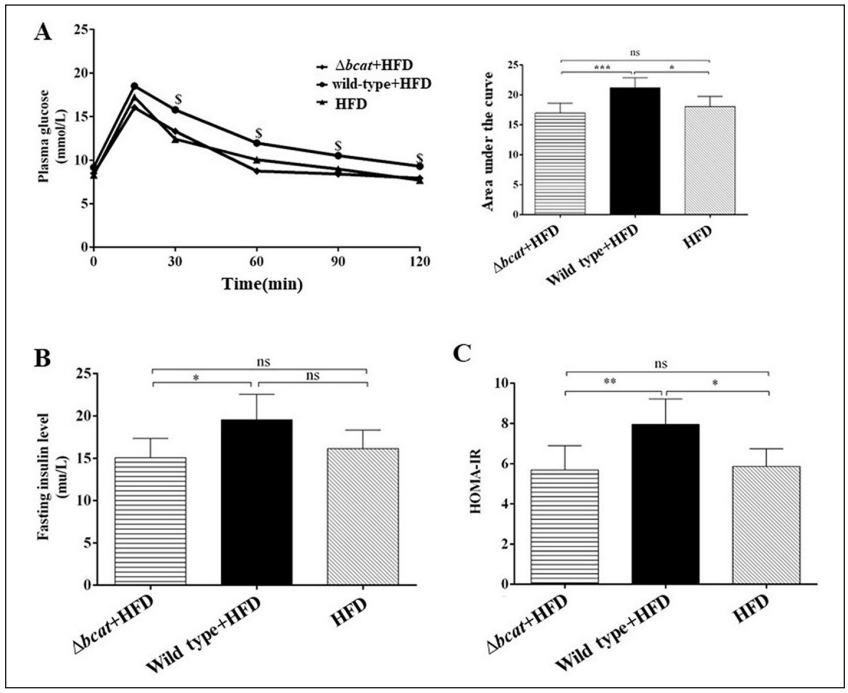


**Figure 5.** Δ*bcat* strain is not able to induce insulin resistance in high-fat fed (HFD) mice. (**A**) Glucose tolerance as reflected by oral glucose tolerance test and area under the curve at the 13th week. (**B**) Fasting insulin levels measured at the 13th week. (**C**) Homeostasis model assessment of insulin resistance (HOMA-IR) calculated by measuring fasting blood glucose and fasting insulin levels in mice. $N = 6$ mice per group. Data are presented as mean ± SD. $^{\$}P < 0.05$ when compared to the HFD group. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.005$. ns, not significant.

These data showed that *bcat* deficiency in *P. gingivalis* did not affect its kinetic growth, biofilm formation, in vivo colonization, and periodontal pathogenicity. In the context of a high-fat diet, *P. gingivalis* aggravated glucose intolerance and insulin resistance together with increased plasma level of BCAAs, while *Δbcat* strain with defective BCAA biosynthesis was not able to induce insulin resistance in mice.

## Discussion

Many epidemiological studies have suggested that periodontitis is a risk factor for insulin resistance-related systemic chronic diseases, such as T2DM and metabolic syndrome (MetS) (Chávarry et al. 2009; Li et al. 2009). A longitudinal study found that the level of blood glucose in diabetes was affected by the persistence of *P. gingivalis* in periodontal pockets of patients afflicted with both periodontitis and T2DM (Makiura et al. 2008). Nonsurgical periodontal therapy alleviated periodontal inflammation and improved insulin sensitivity in patients with T2DM (Navarro-Sanchez et al. 2007). A recent meta-analysis (Daudt et al. 2018) demonstrated an overall odds ratio (OR) of 1.71 for MetS with the presence of periodontitis. An independent intercorrelation between serum level of *P. gingivalis* antibody and MetS was also demonstrated (Iwasaki et al. 2016).

Bhat et al. (2014) found that *P. gingivalis* lipopolysaccharide had significant implications in the development of β-cell compensation and insulin resistance in prediabetes in individuals with periodontitis. Ishikawa et al. (2013) suggested that *P. gingivalis* translocation from the oral cavity to the liver may contribute to the progress of diabetes by influencing hepatic glycogenesis. Arimatsu et al. (2014) showed that repeated oral administration of *P. gingivalis* elicited endotoxemia by altering gut microbiota, thereby inducing systemic inflammation and particularly adipose tissue inflammation and ultimately leading to insulin resistance in mice. In the current study, we also observed aggravated insulin resistance in the HFD-fed mice with periodontal infection of *P. gingivalis*, further supporting the intercorrelation of *P. gingivalis* with insulin resistance.

Many studies (Felig et al. 1969; Guasch-Ferre et al. 2016) have shown that high plasma level of BCAAs is associated with the development of T2DM. A high-BCAA diet can induce insulin resistance in rodents (Newgard et al. 2009). In the current study, we observed an elevated plasma level of BCAAs after periodontal infection with *P. gingivalis*, in parallel to a decreased insulin sensitivity in the HFD-fed mice. More important, by deleting the BCAA biosynthesis-related gene (branched-chain amino acid aminotransferase), we found that the *Δbcat* strain, which showed comparable growth and in vivo colonization but compromised BCAA production relative to the *P. gingivalis* wild-type strain, failed to aggravate insulin resistance in the HFD-fed mice. These data suggest that BCAA biosynthesis is likely the key factor of *P. gingivalis* when inducing insulin resistance.

Of note, our data showed that *P. gingivalis* only aggravated the HFD-induced insulin resistance but had no effect on the insulin sensitivity of NC-fed mice. With the change of people's diet structure, the proportion of high-fat foods in the daily diets increases significantly, which is an important risk factor for the occurrence and development of insulin resistance–related diseases. High-fat diet can increase fasting blood glucose, fasting insulin, and leptin levels, as well as reduce glucose tolerance and insulin sensitivity (Posey et al. 2009). However, it is still inconclusive whether the effect of *P. gingivalis* on insulin sensitivity is related to dietary factors or not. Consistent with our findings, Blasco-Baque et al. (2017) fed the *P. gingivalis*–induced periodontitis mice with either normal or high-fat diet for 3 mo and found that *P. gingivalis* infection had no effect on the insulin sensitivity of mice with a normal diet, but it aggravated the insulin resistance in the high-fat diet group. Watanabe et al. (2008) found that periodontitis aggravated insulin resistance in rats with a high-fat diet, but it had no effect on insulin sensitivity in rats with a normal diet. Conversely, Pulido-Moran et al. (2017) reported no significant correlation between insulin resistance parameters and dietary factors in patients with periodontitis through a cross-sectional study. Taken together, we believe that HFD is the critical factor for the development of insulin resistance, which can be aggravated by the periodontal infection of *P. gingivalis*. However, how diet factors interact with *P. gingivalis* and subsequently counter insulin sensitivity still need further well-controlled studies.

This study for the first time demonstrates that *P. gingivalis* can induce insulin resistance by increasing BCAAs in the blood. However, some cautions should be taken when interpreting data from this study. Although the wild-type *P. gingivalis* and its *Δbcat* strain showed comparable carriage in saliva, their colonization in the periodontal sites needs further investigation. Repeated oral administration of *P. gingivalis* may also affect the composition of intestinal microbiota (Arimatsu et al. 2014; Sato et al. 2017). Intestinal microbiota has been shown to be a major supply of leucine, isoleucine, and valine (Saad et al. 2016; Yoon 2016). We also cannot rule out the possibility that the mice may swallow a sufficient amount of *P. gingivalis* that alters the intestinal microbiota and thus indirectly affects plasma BCAAs. In addition, the adipose tissue inflammation, which is associated with obesity-related insulin resistance (Arimatsu et al. 2014; Blüher 2016), was not examined in the current study. Hence, we could not rule out the possibility that *P. gingivalis* and its *Δbcat* strain may induce distinct adipose tissue inflammation that lead to differed insulin sensitivity. Our results only suggest that *P. gingivalis* aggravates the HFD-induced insulin resistance likely via its BCAA biosynthesis. The effects of *P. gingivalis* on intestinal microbiota and adipose tissue inflammation, as well as the regulation of downstream genes (such as mTOR, S6K-1) in the BCAA-induced insulin resistance signal pathway, warrant further investigations in the future.

## Author Contributions

J. Tian, X. Zhou, X. Xu, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; C. Liu, X. Zheng, X. Jia, contributed to data acquisition and interpretation, critically revised the manuscript; X. Peng, contributed to data acquisition, critically revised the manuscript; R. Yang, contributed to data analysis, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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