

## Title Page

### **8-oxoguanine DNA glycosylase (Ogg1) controls hepatic gluconeogenesis**

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Key words: mtDNA, Ogg1, hyperglycemia, fasting, DNA repair, gluconeogenesis

## Abstract

Mitochondrial DNA (mtDNA) resides in close proximity to metabolic reactions, and is maintained by the 8-oxoguanine DNA glycosylase (Ogg1) and other members of the base excision repair pathway. Here, we tested the hypothesis that changes in liver metabolism as under fasting/feeding conditions would be sensed by liver mtDNA, and that Ogg1 deficient mice might unravel a metabolic phenotype. Wild type (WT) and *ogg1*<sup>-/-</sup> mice were either fed *ad libitum* or subjected to fasting for 24 hrs, and the corresponding effects on liver gene expression, DNA damage, as well as serum values were analyzed. Ogg1 deficient mice fed *ad libitum* exhibited hyperglycemia, elevated insulin levels and higher liver glycogen content as well as increased accumulation of 8oxoG in mtDNA compared to age- and gender matched WT mice. Interestingly, these phenotypes were absent in *ogg1*<sup>-/-</sup> mice during fasting. Gene expression and functional analyses suggest that the diabetogenic phenotype in the *ogg1*<sup>-/-</sup> mice is due to a failure to suppress gluconeogenesis in the fed state. The *ogg1*<sup>-/-</sup> mice exhibited reduced mitochondrial electron transport chain (ETC) capacity and a combined low activity of the pyruvate dehydrogenase (PDH), alluding to inefficient channeling of glycolytic products into the citric acid cycle. Our data demonstrate a physiological role of base excision repair that goes beyond DNA maintenance, and implies that DNA repair is involved in regulating metabolism.

Keywords: mtDNA, gluconeogenesis, Ogg1, mtDNA damage, hyperglycemia, fasting

## 1. Introduction

The base excision repair pathway has been held as the major pathway for removal of oxidized base lesion from the DNA [1]. Base lesions that are substrates for DNA glycosylases may be cytotoxic or premutagenic if left unrepaired. With few exceptions, DNA glycosylases repair both nuclear DNA and mtDNA, and mutants lacking DNA glycosylase function, such as 8-oxoguanine (8oxoG) DNA glycosylase (OGG1) accumulate the corresponding lesion on their DNA and particularly in mtDNA [2].

In support of the premutagenic potential of 8oxoG, double knockout mice lacking OGG1 and MUTYH die prematurely and develop cancer in lung, intestine and liver [3]. MUTYH is a DNA glycosylase that acts in synergy with OGG1 to prevent mutagenesis by removing misincorporating adenine opposite 8oxoG. MUTYH has, like OGG1 also been identified in mitochondrial compartments presumably to protect mtDNA from mutations [4, 5]. In line with the proposed functions of OGG1 and MUTYH, double knockout mice lacking both gene functions accumulate 8oxoG in an age-dependent manner in cancer susceptible tissue [6]. Despite the vulnerability to accumulation of 8oxoG in mtDNA, *Ogg1/Mutyh* double deficient mice exhibit WT mtDNA mutation frequencies in cancer prone tissue. [7]. Thus, the mitochondrial DNA glycosylases seem to be dispensable for protecting mtDNA against mutagenesis and thereby allude to another, unknown role. The biological role of the mitochondrial DNA glycosylase is perhaps illustrated by the metabolic phenotype of the *neil1<sup>-/-</sup>* and *ogg1<sup>-/-</sup>* mice fed on high-fat diet. Both NEIL1 and OGG1 can remove oxidized purines like 8oxoG from the mtDNA, and the corresponding mouse mutants develop obesity, insulin resistance and hyperglycemia. Dysregulation of the metabolism was associated with impaired fatty acid oxidation, tricarboxylic acid cycle genes and peroxisomal proliferator growth factor coactivator 1 $\alpha$  (*Pgc-1 $\alpha$* ) expression upon high fat diet in mutant mice [8, 9]. It has however so far not been possible to attribute the metabolic phenotype to either the mitochondrial or the nuclear isoform of the NEIL1 and OGG1 DNA glycosylases.

Recent discoveries have shown that DNA repair proteins regulate the intracellular redox level that have implications for the metabolism and a specialized form of autophagy: mitophagy [10]. Autophagy is central to the caloric restriction-mediated lifespan extension, mitochondrial fitness, mtDNA integrity and suppression of intracellular oxidative stress [11]. Less is known about the impact of mtDNA repair on fasting-feeding metabolic switches in hepatocytes. Liver mtDNA is highly affected by diet [12], and bioenergetic activation of isolated mitochondria modifies mtDNA in an OGG1-dependent manner [13]. Consequently, gross metabolic switches that involve feeding and fasting are expected to influence mtDNA.

Here, we postulated that the OGG1 DNA glycosylase serves to protect mtDNA from dietary-induced modifications and that a distinct OGG1 deficient phenotype would be manifested during feeding, fasting or alternatively both states.

## 2. Materials and methods

### 2.1. Animals, biochemistry assessment

WT male and female C57BL/6N mice and *ogg1*<sup>-/-</sup> males and females (backcrossed for more than 10 generations) (4 months) were stalled in accordance with the laws and regulations controlling experimental procedures in Norway and the European Union's Directive 86/609/EEC. Fasting was performed by placing mice in cages without food for 24 hrs.

Where indicated, liver from C57BL/6N mice and *ogg1*<sup>-/-</sup> expressing the mitochondrial OGG1 (isoform OGG1- $\alpha$ ); termed “**mtOGG1**” and fed *ad libitum* for 6 months were analyzed. The mice have been described previously [14].

Blood samples were collected by cardiac puncture and sampled in anticoagulant-treated tubes for subsequent plasma preparation. Cells were removed from plasma by centrifugation at 2000g for 10 min and resulting supernatant stored at -80°C for further analysis.

Liver and brain were collected immediately after the animals were sacrificed. One part was stored in ice-cold PBS and immediately used for mitochondrial isolation. Another part was stored in RNA later at 4°C for 24h and homogenized using FastPrep-24 (MP Biomedical, USA).

Blood glucose, plasma insulin and liver glycogen level were determined by using commercially available kits according to the manufacturer's protocol.

### 2.2. Isolation of Nucleic Acids

DNeasy Blood and tissue kit and RNeasy Mini Kit (Qiagen) were used for DNA and RNA isolation, respectively. The concentration was determined by spectrophotometry (Nanodrop spectrophotometer :Thermo Scientific).

### 2.3. LC-MS/MS analysis of 8-oxoguanine

Genomic DNA was digested by incubation with a mixture of nuclease P1 from *Penicillium citrinum* (Sigma, N8630), benzonase (Santa Cruz Biotechnology, sc-391121B) and Alkaline Phosphatase from *E. coli* (Sigma P5931) in 10 mM ammonium acetate buffer pH 6.0, 1 mM MgCl<sub>2</sub> for 40 min at 40°C. The samples were precipitated with ice-cold acetonitrile, supernatants were vacuum centrifuged at room temperature until dry and dissolved in 50  $\mu$ l of water. Quantification was performed with the use of an LC-20AD HPLC system (Shimadzu) coupled to an API 5000 triple quadrupole (ABSciex) operating in positive electrospray ionization mode. The chromatographic separation was performed with the use of an Ascentis Express C18 2.7  $\mu$ m 150 x 2.1 mm i.d. column protected with an Ascentis Express Cartridge Guard Column (Supelco Analytical) with an Exp Titanium Hybrid Ferrule (Optimize Technologies Inc.). The mobile phase consisted of A (water, 0.1% formic acid) and B (methanol, 0.1% formic acid) solutions. The following conditions were employed during chromatography: for unmodified nucleosides –

0.16 mL/min flow, at 25% B for 3.5 min; for 8-oxo(dG) - 0.14 mL/min flow, starting at 5% B for 0.5 min, ramping to 45% B over 8 min and re-equilibrating with 5% B for 5.5 min. For mass spectrometry detection the multiple reaction monitoring (MRM) was implemented using the following mass transitions: 252.2/136.1 (dA), 228.2/112.1 (dC), 268.2/152.1 (dG), 243.2/127.0 (dT), 284.1/168.1 [8-oxo(dG)].

#### 2.4. Gene expression analysis, Gene expression constructs, primers

cDNA was prepared by the cDNA kit provided by Applied Biosystems. Real-time qPCR analyses was performed as described in [11]. The primers are provided in Supplementary Table 1. Taqman probes (Thermo Fisher: Mm01166879 (*Pdk4*) and Mm01208835 (*Pgc-1 $\alpha$* ) were used to assess gene expression in transgenic animals (Figure 6).

Total RNA from three WT and four *ogg1*<sup>-/-</sup> mice from the fed and fasted states were pooled and sent to BGI tech solutions, Hong Kong for sequencing and bioinformatic analysis. Differentially expressed genes (DEGs) were screened with log<sub>2</sub> ratio  $\geq 1$  and false discovery rate FDR < 0.001. Gene ontology enrichment analysis was performed by using hypergeometric test and corrected p-value calculated through Bonferroni correction.

Reference gene for real-time qPCR analyses was  *$\beta$ -Actin*. Results from gene expression analyses were derived from the given number of individual mice, as indicated.

#### 2.5. Cell cultivation

Hepa 1-6 cells were obtained from ATCC and cultivated in “fed conditions”: Dulbecco modified eagle medium (DMEM) with 4.5g/l glucose, 4 mM glutamine, 10% fetal bovine serum (FBS) and 1% penicillin and streptavidin (PS) and “starved conditions”: DMEM with 1g/l glucose, 4mM glutamine, 1% FBS and 1% PS.

For siRNA transfection, 25 nM mouse *Ogg1* siRNA premix and AllStars Negative control siRNA premix obtained from Invitrogen was used.

#### 2.6. Metabolic analysis

Extracellular analyses was performed by Seahorse extracellular analyser (XF24-3), in cultured 50.000 Hep1.6 cells were seeded per well and analysed as described [15], in serum-free DMEM containing 10 mM glucose, 5 mM Pyruvate and 2 mM Glutamine. Extracellular acidification rate (ECAR) was a measure for the glycolytic flux, while Oxygen Consumption rate (OCR) is a measure for hepatocyte respiration. PDH activity was measured after immunocapturing PDH in a colorimetric assay according to the manufacturer's instruction (Abcam; ab109902)

#### 2.7. 8oxoG DNA glycosylase activity analysis

The 8oxoG glycosylase activity was analysed as described previously [14]. Briefly, Mitochondrial cell free extract was prepared by lysing isolated mitochondria in Maltose-Sucrose-Hepes-EDTA/EGTA (MSHE) buffer in 0.5% triton 12 ug mitochondrial extract was incubated with 1 fmol of <sup>32</sup>P-endlabelled 8oxoG-containing oligonucleotide and incubated for 20h at 32°C. The amount of incised oligonucleotide was determined by Phosphoimaging after separating single stranded oligonucleotides on a denaturing PAGE.

#### 2.8. Mitochondrial electron transport chain (ETC) complex I, II and V activity.

Mitochondrial complex activities were measured, slightly modified from Barrientos [7].

#### 2.9. Statistical Analysis

Two-way ANOVA with Sidak's multiple comparisons test was employed to evaluate statistical significant differences between the two genotypes on fed/fasting alterations. Holm-Sidak multiple T-test was used to calculate significant differences between two genotypes during the fed condition, and one-way ANOVA with Tukey's multiple comparisons test was used to evaluate differences in expression of *Pdk4* and *Pgc-1α* in four different mouse cohorts. \*p<0.05.

### 3. Results

#### 3.1. *Ogg1* is required for repair of genomic 8oxoG during feeding but not upon fasting

In order to investigate the responsiveness of *Ogg1* to metabolic states in the liver, WT and *ogg1*<sup>-/-</sup> mice were either fed *ad libitum* or alternatively kept without food for 24 h and analyzed for liver *Ogg1* expression and OGG1 DNA glycosylase activity. *Ogg1* expression was two-fold higher in the fasted state, compared to the fed state (Figure 1A). In parallel with the gene expression, the mitochondrial OGG1 activity was higher during fasting than in the fed state (borderline significance: p=0.052) (Figure 1B and C). Thus, OGG1 is increased during fasting. *Ogg1* has previously been shown to protect against accumulation of mitochondrial 8oxoG accumulation in *ad libitum* fed mice [2]. To evaluate the effect of the metabolic states on nuclear versus mitochondrial genome integrity, 8oxoG levels were analyzed in total DNA as well as in mtDNA extracted from isolated mitochondria. There was no significant difference in genomic 8oxoG levels in fed or fasted animals, or between WT and *ogg1*<sup>-/-</sup> mice (Figure 1D). However and as reported previously, mtDNA from the fed *ogg1*<sup>-/-</sup> contained significantly higher levels of 8oxoG than mtDNA from the fed WT mice. Interestingly, there was no significant difference between 8oxoG levels in mtDNA from fed and fasted WT mice, as well as between 8oxoG from fasted WT and *ogg1*<sup>-/-</sup> mtDNA (Figure 1E). These results support that OGG1 is particularly important to the maintenance of mtDNA integrity, and especially during feeding conditions.

#### 3.2. *Ogg1* deficiency manifests in metabolic dysfunction, selectively during feeding conditions

In view of the distinct effect of fasting and feeding on the genome integrity of the different genotypes, corresponding biomarkers for each state were assessed in both genotypes/metabolic states. Previously, *ogg1*<sup>-/-</sup> mice were found to be more sensitive to high fat diet-induced metabolic syndrome than WT [9]. Here, *ogg1*<sup>-/-</sup> mice displayed hyperglycemia even upon normal diet, which correlated with increased insulin level (Figure 2A and B). Interestingly, fasting lowered blood glucose in both genotypes to the same level. In correlation to this, insulin was also reduced to similar levels. Liver glycogen in fed *ogg1*<sup>-/-</sup> mice tended to be slightly elevated but not significant, whereas fasting abolished the apparent difference (Figure 2C). It is therefore concluded that these metabolic biomarkers demonstrate that the OGG1 deficient phenotype is manifested under feeding conditions but not during fasting. Importantly, the metabolism-associated phenotype was not observed in brain (Supplementary Figure 1). Neither expression of OGG1 nor its enzymatic activity was significantly affected by fasting (Supplementary Figure 1A, B). Similar to liver, genomic 8oxoG in the brain was not significantly altered in fed or fasted, WT and *ogg1*<sup>-/-</sup> mice (Supplementary Figure 1C).

#### 3.3. *Ogg1* deficiency renders mice less responsive to dietary-induced gene activations

Gene expression profiling confirmed the relatively subtle effect of *Ogg1* deficiency during fasting as compared to *ad libitum* feeding (Figure 3). While more than 2000 genes were differentially expressed in the *ogg1*<sup>-/-</sup> compared to WT in the fed state, the same number reduced to less than 800 during fasting. The analyses additionally showed that *Ogg1* renders the liver more responsive to the fed-fasting switch (more than 2600 genes in WT versus less than 700 in the *ogg1*<sup>-/-</sup> were affected (Figure 3A). Gene ontology analyses demonstrated that metabolism-associated genes were abundant among the differentially expressed genes. (Figure 3B).

### 3.4. Suppression of gluconeogenesis in the fed state requires OGG1

To follow up on the fed-state dependence of the *ogg1*<sup>-/-</sup> phenotype, the expression of gluconeogenesis genes were assessed in more detail. *Glut2* encodes a glucose transporter that is suppressed during feeding in an insulin-independent manner. *Fructose biphosphatase 2 (Fbp2)*, *glucose 6-phosphatase (G6P)* and *Pyruvate dehydrogenase kinase 4 (Pdk4)* are key genes in gluconeogenesis and their expressions are suppressed upon feeding. Interestingly, the expression of *Fbp2*, *Pdk4* and *Glut2* was higher in *ad libitum*-fed *ogg1*<sup>-/-</sup> mice compared to WT mice (Figure 4A) and implied a defective suppression of gluconeogenesis in the fed state. Activation of hepatic pyruvate dehydrogenase (PDH) is essential to shuttle glycolytic products into the citric acid cycle under feeding. Importantly, the PDH activity was lower in fed *ogg1*<sup>-/-</sup> mice (Figure 4B), in coherence with a deficient regulation of glycolysis versus gluconeogenesis. In comparison, fasting increased expression of the gluconeogenetic genes to similar levels in WT and *ogg1*<sup>-/-</sup> mice (Supplementary Figure 2A). Furthermore, fasting reduced PDH activity approximately 5-fold in WT mice, but had no effect in *ogg1*<sup>-/-</sup> mice (Supplementary Figure 2B). *Pgc-1 $\alpha$*  was significantly elevated in *ogg1*<sup>-/-</sup> mice as well (Figure 4A), indicating that gluconeogenesis is indeed activated during feeding in *ogg1*<sup>-/-</sup> mice [16].

### 3.5. Reduced mitochondrial function in the absence of *Ogg1*

The lower PDH activity in the fed state in *ogg1*<sup>-/-</sup> mice could be indicative of an insufficient ability of the mitochondria to process incoming acetylCoA from the glycolysis. In order to investigate whether low mitochondrial activity could be a plausible explanation for this, the ETC capacity of the mitochondria was assessed. Complex I, II and V were selected because they represent either mixed assemblies of nuclear and mtDNA-encoded subunits (complex I and V) or only nuclear DNA-encoded subunits (complex II). Complex I is the largest complex of 44 subunits, and most frequently affected in cases of mitochondrial diseases, while complex V may be rate-limiting for OXPHOS [17]. Importantly, complex I, II and V activities were all significantly lower in *ad libitum* fed *ogg1*<sup>-/-</sup> mice compared to matching controls (Figure 5A). The reduction was stoichiometric in complex I, V and II (-33%), which is indicative of a general suppression of mitochondrial function rather than a selective effect of mtDNA disintegrity. Fasting caused a general suppression of mitochondrial ETC activity and to a comparable extent in both genotypes (Supplementary Figure 3). Together, these data imply that mitochondrial activity is reduced in *ogg1*<sup>-/-</sup> mice.



In order to examine the impact of *Ogg1* on glycolytic flux, a cellular system was chosen where *Ogg1* expression was manipulated by siRNA-mediated knockdown. Murine hepatocytes (Hep1.6 cells) were grown in glucose rich (mimicking fed conditions) and the glycolytic flux was analyzed by Seahorse extracellular flux analyses. siOgg1 transfection reduced expression of *Ogg1* by 50% (Supplementary Figure 4A). The suppression of *Ogg1* resulted in a significant dampening of the extracellular acidification rate (ECAR) (Figure 5B), which is an indirect measure of lactate production, and thereby demonstrated that glycolytic flux was lowered in *Ogg1*-depleted hepatocytes. In contrast, oxygen consumption rate (OCR) was not affected by the *Ogg1* expression level under these cell cultivation conditions (Supplementary Figure 4B). When Hep1.6 cells were tested for starvation-mimicking conditions (low glucose/serum deprived), there was no effect of siOgg1 transfection on ECAR or OCR values (Supplementary Figure 4C, D), again demonstrating the specific metabolic niche of OGG1.

In view of OGG1's documented role in maintaining mtDNA in the fed state (Figure 1), we investigated the particular role of mitochondrial OGG1 on metabolic regulation *in vivo*. WT and *ogg1*<sup>-/-</sup> mice that overexpress a mitochondrially targeted OGG1 (or a control vector) were fed *ad libitum* for 6 months prior to gene expression analyzes of *Pdk4* and *Pgc-1 $\alpha$* . *Pdk4* expression was elevated in *ogg1*<sup>-/-</sup> mice expressing only control vector (Figure 6), confirming the result with non-transgenic mice (Figure 4). Overexpression of mitochondrial OGG1 did however not significantly change this situation, as the overexpressing WT and *ogg1*<sup>-/-</sup> mice displayed average *Pdk4* levels that were between vector-expressing WT and *ogg1*<sup>-/-</sup> mice. There was little effect of *Ogg1* deficiency on the *Pgc-1 $\alpha$*  expression level in these 6 months animals, except that mitochondrial OGG1 showed increased expression in WT background (Figure 6). We conclude that mitochondrial OGG1 needs to be fine tuned for proper gluconeogenic regulation.

#### 4. Discussion

In this study the role of (mt)DNA repair on metabolic regulation has been addressed by focusing on the genotoxicity, gene expression and functional effect in *ogg1*<sup>-/-</sup> mice subjected to either feeding or fasting conditions. Importantly, gross accumulation of genomic 8oxoG in liver is not a general characteristic of repair deficiency in the KO. On the other hand, the increased level of 8oxoG in mtDNA suggests that the cellular role of OGG1 in liver is primarily attributed to maintaining mtDNA. Measures of DNA integrity and gene expression analyses implied that the cellular role of OGG1 is predominantly relevant to feeding conditions rather than during fasting. Under feeding, OGG1 contributes to efficient hepatic glycolysis combined with inhibition of gluconeogenesis.

The observation that base excision repair mutants are metabolically compromised is not new. Previously it was reported that oral glucose tolerance was reduced in *ogg1*<sup>-/-</sup> [9], and that a high fat diet increased hyperglycemia in *neil1*<sup>-/-</sup> [8]. The novelty herein lies in the metabolism-dependent phenotypes including mtDNA integrity, gene expression and hyperglycemia. Although the detailed mechanism underlying the mtDNA repair-mediated regulation of

gluconeogenesis was not unravelled in this study, intracellular cAMP signaling is potentially involved. In a separate study of cultured neurons, mitochondrial ROS induced by glutamate exposure mediated the increase in expression of the Ape1 AP endonuclease in a CREB-dependent manner [18]. CREB is strongly implicated in regulation of gluconeogenesis [19]. It is also possible that the mtDNA repair enzymes are directly involved in regulating glycolysis/gluconeogenesis [20]. It is worth noting that the increase in *Pgc-1 $\alpha$*  expression upon overexpression of mitochondrial OGG1 (Figure 6) alludes to a coupling between mtDNA processing and metabolism that is likely to involve this central transcriptional coactivator.

Here, we also show for the first time that mitochondrial ETC capacity is compromised in the *ogg1<sup>-/-</sup>* liver. This is in contrast to earlier report of *ogg1<sup>-/-</sup>* mitochondrial capacity [21]. The explanation for this discrepancy is probably that the cited report pooled samples from 2 to 14 months old individuals, whereas we specifically used 4 months old, backcrossed animals. We have separately found that mitochondrial function varies in an age-, complex- and tissue-specific manner between WT and *ogg1<sup>-/-</sup>* mice (unpublished results). The lower PDH activity and decreased mitochondrial ETC activity in *ogg1<sup>-/-</sup>* liver may be indicative of a dampened mitochondrial metabolism in the *ogg1<sup>-/-</sup>* hepatocyte. It is therefore interesting that the primary effect of mitochondrial dysfunction in hepatocytes (as in *ogg1<sup>-/-</sup>*) seems to be an impaired ability to perform glycolysis during feeding.

Genomic 8oxoG levels were not significantly different between the genotypes, which implies that OGG1 is dispensable for accumulation of 8oxoG *in vivo*. In mitochondria, the impact of OGG1 on mtDNA suggest that base excision repair is restricted to the feeding (glycolytic) metabolism in liver. As an analogy, it is known that DNA synthesis is restricted to the reductive (glycolytic) phase of the cell cycle [22]. Although OGG1 was upregulated during fasting and thereby indicates a stronger effect of OGG1 deficiency than in the feeding state, it is possible that the upregulation is intended to increase the capacity for repair of accumulating DNA damage during fasting once the hepatocytes reenter the feeding/glycolytic phase. Prolonged fasting (3 days) is known to induce DNA damage [23]. Caloric restriction causes reduced oxidative stress and decreased 8oxoG in mtDNA [24] and both mitophagy and DNA repair factors are involved in this process [25]. Thus, it is possible that caloric restriction and shorter fasting periods exert similar effects on liver DNA which are tolerable, and even beneficial, for mtDNA integrity and proper liver metabolism.

Except for the use of transgenic mice expressing a mitochondrially targeted OGG1, we did not specifically address the mitochondrial role of OGG1 in this study. It is unlikely that the mitochondrial dysfunction observed in *ogg1<sup>-/-</sup>* mice is caused by increased mutagenesis resulting from accumulating premutagenic 8oxoG lesions. First, complex II activity, which is entirely nuclear encoded, was reduced to the same extent as complex I and V. Second, we previously found that *Mutyh<sup>-/-</sup>/ogg1<sup>-/-</sup>* double knockout animals displayed normal mtDNA mutation frequency [7], despite the carcinogenic impact of nuclear DNA mutagenesis in these animals [3]. Rather, we favor a putative role for OGG1 in regulating mitochondrial metabolism via its

interaction with mtDNA. In a recent study, we discovered that modifications on the mtDNA formed a distinct pattern that was representative of the metabolic activity of the specific mitochondria type [13]. The pattern of mtDNA modifications was not only different in *ogg1*<sup>-/-</sup> mitochondria, but the induced accumulation of such modifications upon energization of isolated mitochondria *in vitro* was also dependent on OGG1. These observations motivate us to speculate that an OGG1-mtDNA interaction plays a regulatory role in mitochondrial metabolism.

We have not investigated whether these findings can be applied to the human situation. Two isoforms of OGG1 exist in the mitochondria of human cells: the  $\alpha$ - and  $\beta$ - form. The latter is mitochondria specific, and lacks DNA glycosylase activity [26]. The mitochondrial form of OGG1 needs to be translocated across the mitochondrial membrane and this translocation has been shown to be inefficient in old individuals [27]. The common Ser326Cys SNP has been associated with impaired insulin sensitivity and type II Diabetes [28, 29]. The Ser326Cys polymorphism is associated with impaired catalytic activity and reduced intracellular trafficking.

## **5. Conclusion**

In summary, we show for the first time that OGG1 plays a distinct role in regulation of blood hepatic glucose production. The function is coupled to the ability to suppress gluconeogenesis during feeding. The study shows that DNA repair has implications beyond DNA maintenance, and implies that a sufficient DNA repair capacity is essential to avoid hyperglycemia. Conditions that stimulate OGG1 should therefore be evaluated for treatment of type II Diabetes.

## **Acknowledgments**

We are indebted to Rajikula Suganthan for animal support.

## **Funding**

This project was supported by grants from University of Oslo to LE, and from the Norwegian Financial Mechanism 2009-2014 and the Ministry of Education, Youth and Sports under Project Contract no. MSMT-28477/2014. The PROMEC proteomics and metabolomics core facility is supported by the Liaison Committee between the Central Norway Regional Health Authority (RHA) and the Norwegian University of Science and Technology (NTNU) (grant no: 46040500).

## **Conflict of Interest.**

The authors declare no potential conflicts of interest.

## **Supplementary data**

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## Figure legends

**Figure 1 Fasting increases *Ogg1* gene expression and activity but unaltered 8-oxoG level in liver of *Ogg1*-deficient mice.** WT and *ogg1*<sup>-/-</sup> mice were fasted for 24 hours. (A) *Ogg1* mRNA expression in liver (B) Quantification of 8-oxoG incision activity and (C) representative image of 8-oxoG incision activity in mitochondrial extract from ad libitum fed WT (Fed), fasted WT for 24 hr (Fasted), fasted *ogg1*<sup>-/-</sup> (*Ogg1*<sup>-/-</sup>). “negative” and “positive” indicate buffer only, and recombinant OGG1 (10ng), respectively. (D) Genomic 8-oxoG level in liver as evaluated by mass spectrometry. (E) 8-oxoG level in liver mtDNA as evaluated by mass spectrometry. Data are presented as mean ± SEM (N=6-8), or as median with range \*p<0.05.

**Figure 2 Increased plasma glucose and insulin in *ogg1*<sup>-/-</sup> mice in the fed state. WT and *ogg1*<sup>-/-</sup> mice were fasted for 24 hours.** (A) Plasma glucose level (B) Plasma insulin level and (C) liver glycogen level. Data are presented as mean ± SEM (N=6-8), \*p<0.05.

**Figure 3 RNA sequencing reveals mostly upregulated differentially expressed genes (DEGs) associated with metabolic pathways and transcriptional activity in liver of fed *Ogg1*-deficient mice.** (A) DEGs that are up- and downregulated in liver of *ogg1*<sup>-/-</sup> mice as compared to WT and in fasted as compared to fed mice (B) Top ten KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways that are significantly enriched in DEGs of fed *ogg1*<sup>-/-</sup> as compared to fed WT liver.

**Figure 4 *Ogg1* deficiency alters hepatic gene expression and decreases pyruvate dehydrogenase activity in the fed state.** WT and *ogg1*<sup>-/-</sup> mice (4 months of age) were fed *ad libitum*. (A) mRNA expression of glucose transporter 2 (*Glut2*), fructose-bisphosphatase 2 (*Fbp2*), glucose-6-phosphatase (*G6Pase*), pyruvate dehydrogenase kinase 4 (*Pdk4*) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*Pgc1α*) (B) Activity of liver pyruvate dehydrogenase (PDH). Data are presented as mean ± SEM (N=6-8), \*p<0.05.

**Figure 5 Mitochondrial dysfunction and impaired glycolysis in the absence of OGG1.** (A) Liver mitochondria from ad libitum fed wt and *ogg1*<sup>-/-</sup> mice (4 months) were analyzed for ETC biochemical complex activity. (N=7-8) (B) Glycolytic flux was measured in cultivated hepatocytes (Hep1.6) transfected with siRNA for control or siOgg1. Glycolytic flux was assessed as extracellular acidification rate, measured by Seahorse analyses. Data are presented as mean ± SEM from three independent experiments \*p<0.05.

**Figure 6. The gluconeogenic genes *Pdk4* and *Pgc-1α* respond to mtOGG1, dependent on *Ogg1*.** *Pdk4* and *Pgc-1α* expression in liver from mtOGG1 transgene animals of WT and *ogg1*<sup>-/-</sup> background that have been fed ad libitum for 6 months. Data are presented as mean ± SEM; (For WT, wt-mtOgg1, mtOGG1- *ogg1*<sup>-/-</sup> N=3 and for *ogg1*<sup>-/-</sup> N=4; \*p<0.05.

Figure 1

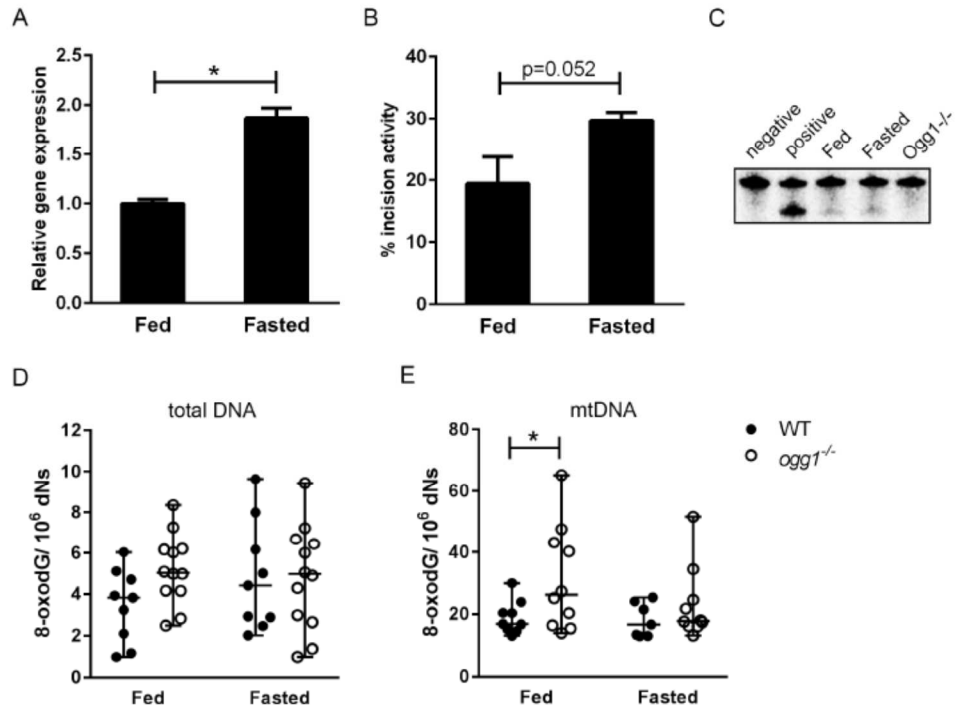




Figure 2

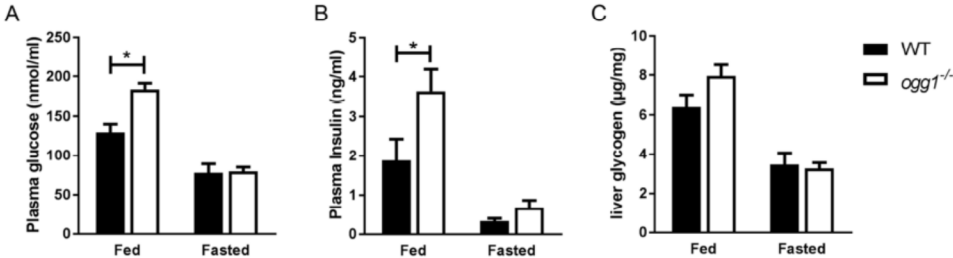


Figure 3

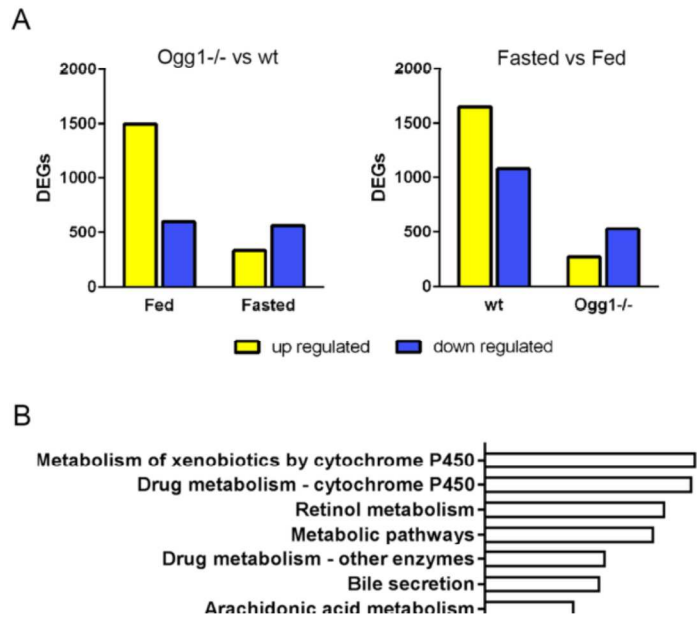
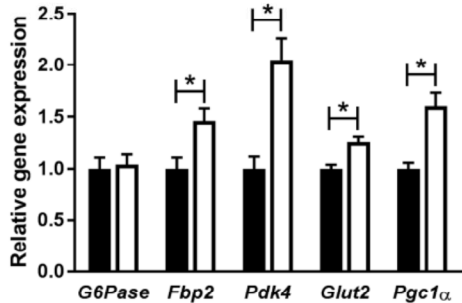


Figure 4

A



B

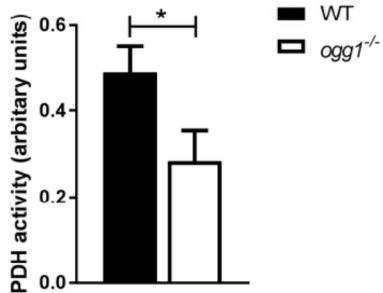


Figure 5

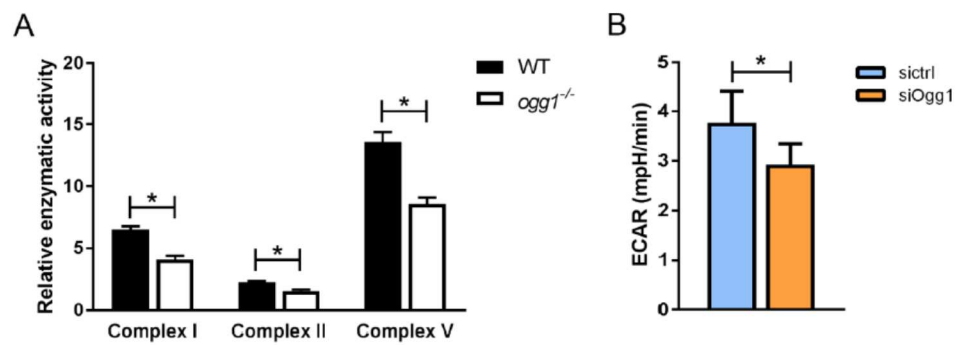


Figure 6

