Application Title: *Determining the roles of serine and glycine in insulin resistance.*

Principal Investigator: Dr. Michal K Handzlik

1. Project Accomplishments:

The major goal of this project was to develop a stable isotope tracer infusion method to quantitatively determine the impact of diabetes and morbid obesity on serine and glycine metabolism in a mouse model of diabetes/obesity. The second aim of this project was to utilize dietary interventions to assess the metabolic consequences of serine or glycine starvation on the development of adiposity and *de novo* lipid biosynthesis. The project has also provided funding to attend The Glucose Clamping The Conscious Mouse: A Laboratory Course, and to learn catheterization technique in order to continuously infuse [U-^{13}C]glucose, [U-^{13}C]serine, and [U-^{13}C]glycine into wild-type and *db/db* mice to quantify whole-body sources and fluxes of serine and glycine in the context of diabetes and obesity. In addition to a successful establishment of the stable isotope tracer infusion approach, this project successfully developed alterative and discriminatory serine tolerance test (STT) to quantify whole-body serine clearance which may ultimately serve as a diagnostic tool to screen for patients particularly susceptible to aberrant serine homeostasis. Finally, as part of the STT, the other major accomplishment of this project was identification of key tissues and metabolic pathways, using [U-^{13}C]serine, of serine disposal.

2. Specific Aims:

Specific Aim 1. Identify and quantify sources and sinks of serine and glycine in obesity and diabetes.

Low circulating serine and glycine are hallmarks of metabolic disorders[1-3], yet how and why obesity and type 2 diabetes (T2D) impact serine and glycine metabolism across tissues is poorly defined. To explore this relationship in more detail, we quantified serine, glycine, and methionine in an established mouse model of morbid obesity and hyperglycaemia driven by leptin-receptor deficiency, *db/db* mice on Black Kaliss (BKS) background, comparing results to age-matched wild-type (WT) controls. Relative to WT, fasting *db/db* mice exhibited a ~30% reduction in serine levels within the liver and kidney (Fig. 1a-b). Glycine abundance, which is 5-10-fold greater than serine in tissues, was also reduced by ~30-50% in liver, kidney, inguinal white adipose tissue (iWAT), and plasma (Fig. 1a-d). Methionine is linked with serine metabolism via the one-carbon pathway, and its levels were also reduced in liver, iWAT, and plasma (Fig. 1a, c-d). Collectively, these results indicate that obesity/diabetes is associated with lower levels of serine and glycine in tissues important for glucose and lipid homeostasis.

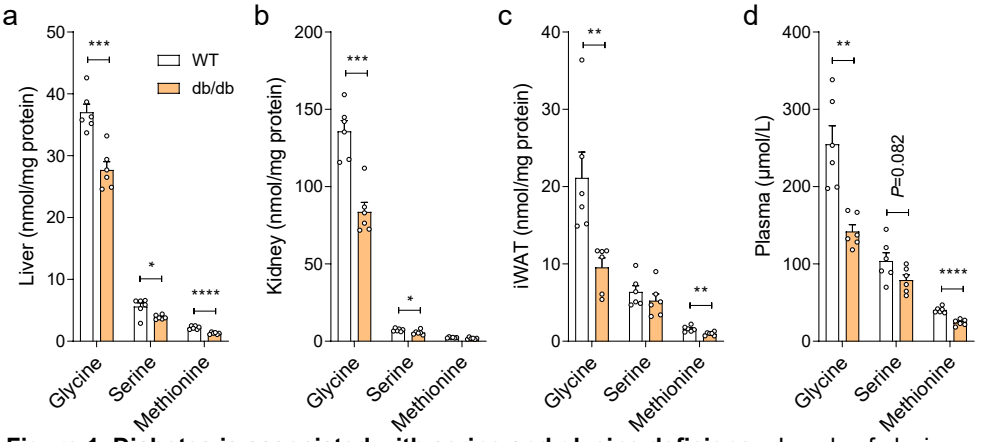

Figure 1. Diabetes is associated with serine and glycine deficiency. Levels of glycine, serine, and methionine in wild-type (WT) and BKS-*db/db* mice in the liver (a), kidney (b), inguinal white adipose tissue (c), and plasma (d) after a 6-hour fasting. Data were tested using an independent t-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Next, to quantify whole-body serine and glycine fluxes, WT and *db/db* mice had their jugular veins catheterized, and [U-^{13}C]glucose, [U-^{13}C]serine, and [U-^{13}C]glycine were independently infused for 3 hours after 6-hour fasting at rates corresponding to ~10% plasma metabolite enrichment in order not to perturb

systemic metabolism. Given higher body weight and fat content of *db/db* mice (Fig. 2a-b), we decided to infuse [U-^{13}C]glucose, [U-^{13}C]serine, and [U-^{13}C]glycine per gram of lean mass to avoid the potentially confounding factor of excessive adipose tissue in *db/db* mice. Previous studies in diabetic humans have demonstrated that infusion of [U-^{13}C]glucose per gram of lean mass leads to the same metabolic conclusions when glucose is infused per total body mass[4,5].

Following a 3-hour infusion of [U-^{13}C]glucose, plasma glucose enrichment in WT mice reached a plateau of ~14% (Fig. 2d). Considering that *db/db* mice have elevated circulating blood glucose levels relative to WT (Fig. 2c), [U-^{13}C]glucose infusion per lean mass led to a lower plasma glucose enrichment in *db/db* mice (Fig. 2d). This in turn translated into 2.3-fold higher rate of glucose appearance (Ra) into the circulation relative to WT (Fig. 2e). Likewise, calculated whole-body gluconeogenic flux was significantly higher in *db/db* mice in comparison with WT mice (Fig. 2f). Collectively, this data demonstrates that we could successfully deliver ^{13}C-tracer into WT and *db/db* mice generating expected and previously described metabolic alterations in a mouse model of obesity and diabetes.

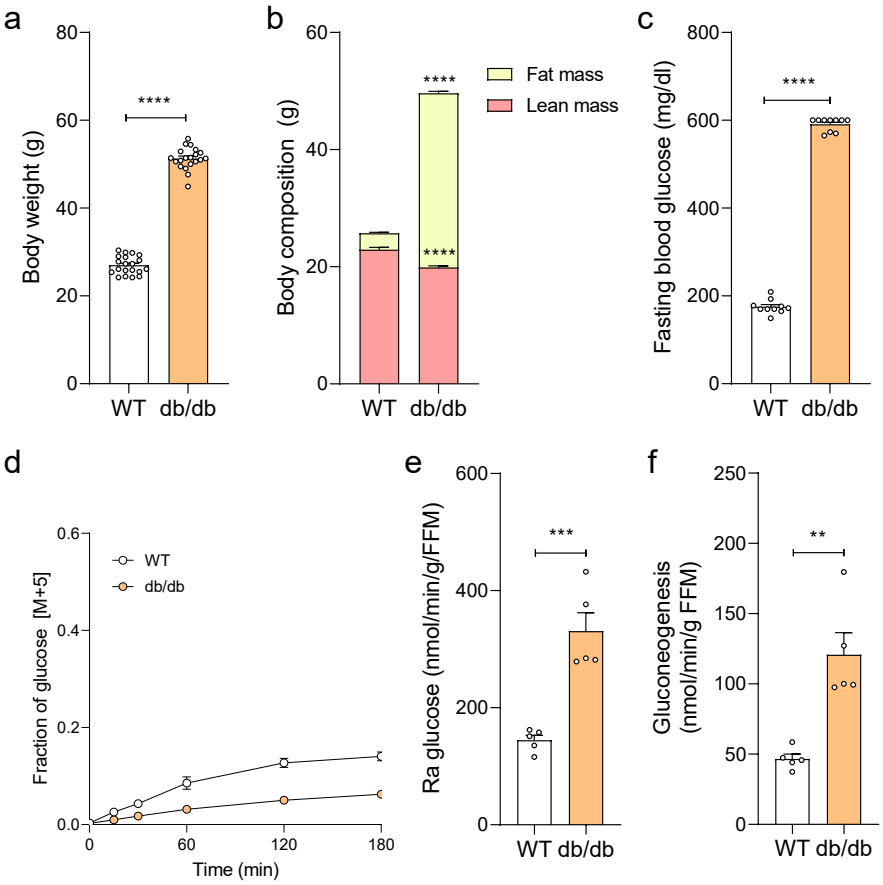

Figure 2. BKS-*db/db* mice have higher rate of whole-body glucose appearance (Ra) and gluconeogenesis. Body weight (a), body composition (b), fasting blood glucose (c), time-course of plasma glucose enrichment (d), rate of glucose appearance (Ra; d), and absolute whole-body gluconeogenic flux (f) in WT and *db/db* mice following a 3-hour infusion with 200 mM [U-^{13}C]glucose at a rate of 0.1 µl/min/g FFM. Data were tested using an independent t-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

To determine contribution of glucose into different metabolic pools, we utilized gas chromatography mass spectrometry (GC-MS) and quantified enrichment of glycolytic intermediates, amino acids, and TCA cycle intermediates to gain insights into the contribution of circulating glucose into synthesis of these metabolites (Fig. 3). To account for differences in plasma [U-^{13}C]glucose enrichments between WT and *db/db* mice, we normalized metabolite enrichment by plasma [U-^{13}C]glucose enrichment in individual animals as previously described[6]. As

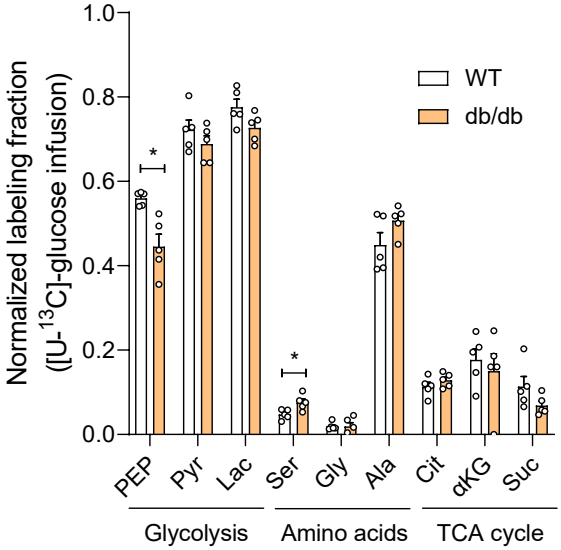

Figure 3. Normalized plasma metabolite enrichment following a 3-hour infusion of [U-^{13}C]glucose in WT and *db/db* mice. Plasma enrichment of glycolytic intermediates, amino acids, and TCA cycle intermediates was normalized to circulating glucose. Data were tested using an independent t-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

expected, following a 3-hour [U-^{13}C]glucose infusion, normalized plasma phosphoenolpyruvate, pyruvate, and lactate enrichments in WT mice were ~60-80%, indicating that the vast majority of circulating glycolytic intermediates comes from glucose. Interestingly, although enrichment of normalized plasma alanine and TCA cycle intermediates reached ~45% and 12%, respectively, normalized serine and glycine enrichments were on average 4.7% and 1.5%, respectively. This data indicates that glucose is not a major carbon source of circulating serine and glycine, at least under current experimental conditions.

In mammals likely sources of circulating and tissue serine may be derived from glucose, glycine and/or protein breakdown. Although concentration of circulating blood glucose is ~5 mM, our data demonstrate that glucose is unlikely to be a major source of circulating serine. Therefore, we next tested the hypothesis that glycine, whose circulating concentration is ~3 times as high as serine (~300 μM vs. 100 μM)[1], may provide carbon via serine hydroxymethyltransferase (SHMT) to synthesize serine. To test this hypothesis, we infused [U-^{13}C]glycine for 3 hours to ensure an isotopic steady-state into WT and BKS-*db/db* mice following a 6-hour fasting (Fig. 4). While plasma glycine enrichment reached a plateau within 60 min in both genotypes, glycine contribution toward serine enrichment was increased in BKS-*db/db* mice, indicating that diabetes and obesity accelerate conversion of glycine to serine. Collectively, this data demonstrates that glycine may serve as a serine pool to buffer altering serine availability during metabolic stress.

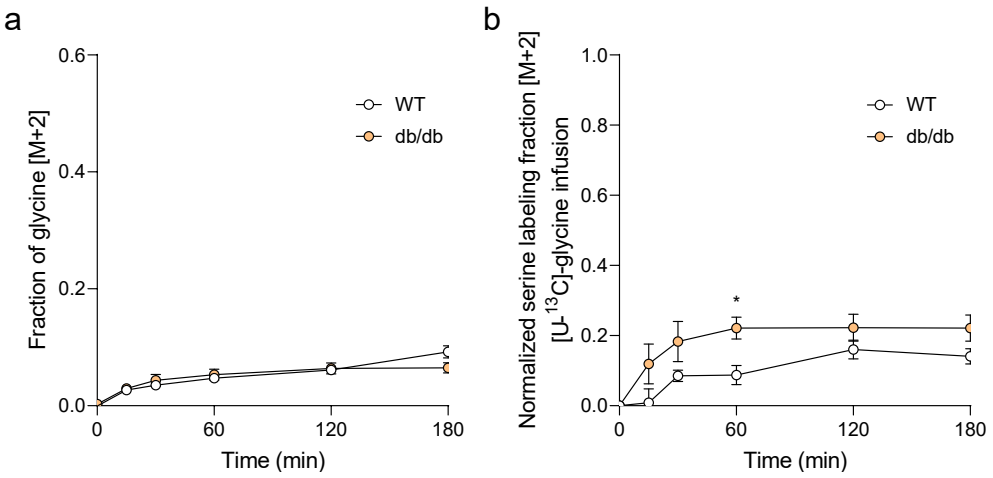

Figure 4. Time-course of plasma glycine enrichment (a) and plasma serine enrichment (b) during infusion of [U-^{13}C]glycine in WT and *db/db* mice. Data are mean ± SEM and were tested using an independent t-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Using steady-state enrichment data for glucose, serine, and glycine, we next calculated rates of appearance of glucose, serine, and glycine in WT and BKS-*db/db* mice to explore how obesity and diabetes impact whole-body turnover fluxes (Fig. 5). Consistent with previous observations, Ra for serine and glycine was substantially lower compared to glucose in WT mice[6]. Moreover, in contrast to our hypothesis there were no differences in steady-state serine and glycine turnover fluxes between WT and BKS-*db/db* mice, at least under current experimental conditions, indicating that obesity and diabetes do not impact whole-body serine and glycine fluxes.

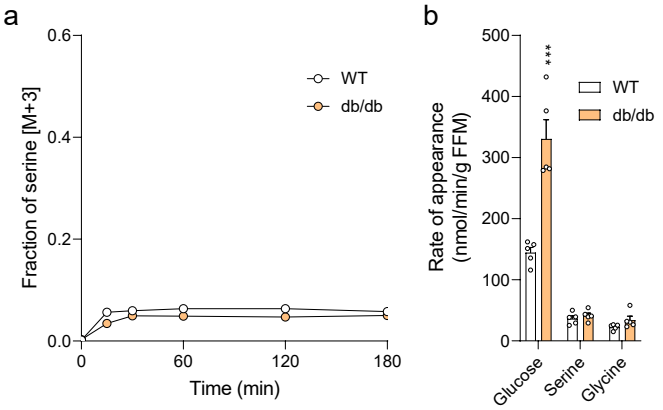

Figure 5. Calculated rate of appearance (Ra) of glucose, serine, and glycine in WT and *db/db* mice. Plasma serine enrichment (a) and rate of metabolite appearance (b) following a 3-hour infusion of corresponding tracers in WT and *db/db* mice. Data are mean ± SEM and were tested using an independent t-test. *** $P < 0.001$.

Circadian and post-prandial variations in amino acids (or glucose) make diagnosis of metabolic defects challenging. We therefore hypothesized that utilization of a “serine-tolerance test” (STT) might better gauge serine/glycine/one carbon (SGOC) metabolism in animals and identify subjects with elevated serine disposal. Therefore, we turned to develop and optimize a STT. Applying dosages used in human clinical trials (400 mg/kg)[7], we orally administered serine to fasted C57BL6/J (WT) mice and quantified plasma amino acid pharmacokinetics to gauge the dynamics of serine

clearance (Fig. 6). Of the three different doses tested, we found that 400 mg/kg dose sufficiently perturbed serine homeostasis to be useful as a diagnostic and screening tool, yet plasma serine returned to near-baseline values within 2 hours of serine administration.

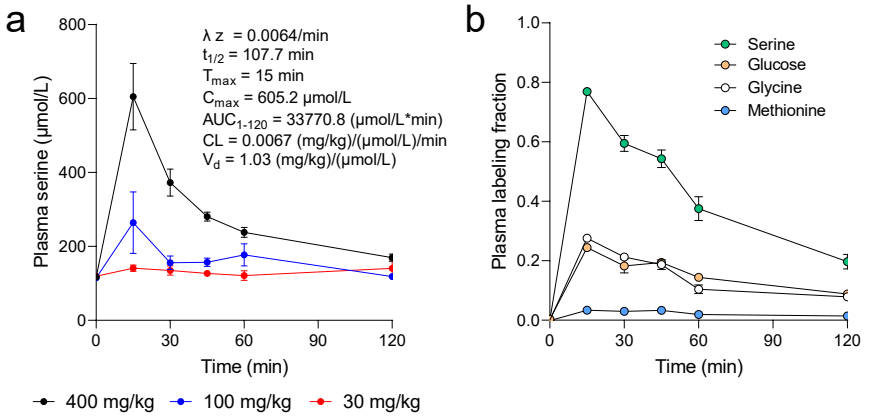

Figure 6. Development of a serine tolerance test. Plasma serine pharmacokinetics following oral administration of three different doses of serine in C57BL6 mice (a), and time course of quantitative determination of a metabolic fate of serine using [U-^{13}C]serine administered via oral gavage at a dose of 400 mg/kg. Data are mean ± SEM.

To identify the most critical pathways involved in serine disposal, we next orally administered [U-$^{13}C_3$]serine to overnight fasting WT mice and quantified enrichment of downstream metabolites (Fig. 6b). Plasma serine enrichment peaked at 15 min post-oral gavage, consistent with plasma serine levels in our preliminary work and reached levels of just below 80%, steadily falling to ~20% 2 hours after [U-^{13}C]serine administration. GC-MS-quantified contribution of serine into potential downstream metabolites revealed that glucose was labeled at similar levels to glycine throughout the test.

We next explored downstream serine metabolism in ten different organs considered relevant for serine homeostasis. Carbon from oral [U-$^{13}C_3$]serine was robustly incorporated into glycine, pyruvate, and citrate within the fasting liver relative to other organs (Fig. 7a-d), demonstrating that hepatic gluconeogenesis is a major pathway for serine disposal in some contexts. To test whether insulin resistance-induced dysregulation of gluconeogenic flux drives accelerated serine disposal, we delivered both glucose (2 g/kg) and serine (400 mg/kg) to fasted WT and *db/db* mice. When comparing serine tolerance in these cohorts , we observed a significant reduction in AUC_{SER} in *db/db* mice, despite administering ~2-fold more serine (Fig. 8). On the other hand, there were no differences in AUC_{SER} between WT and *db/db* mice when serine was administered alone.

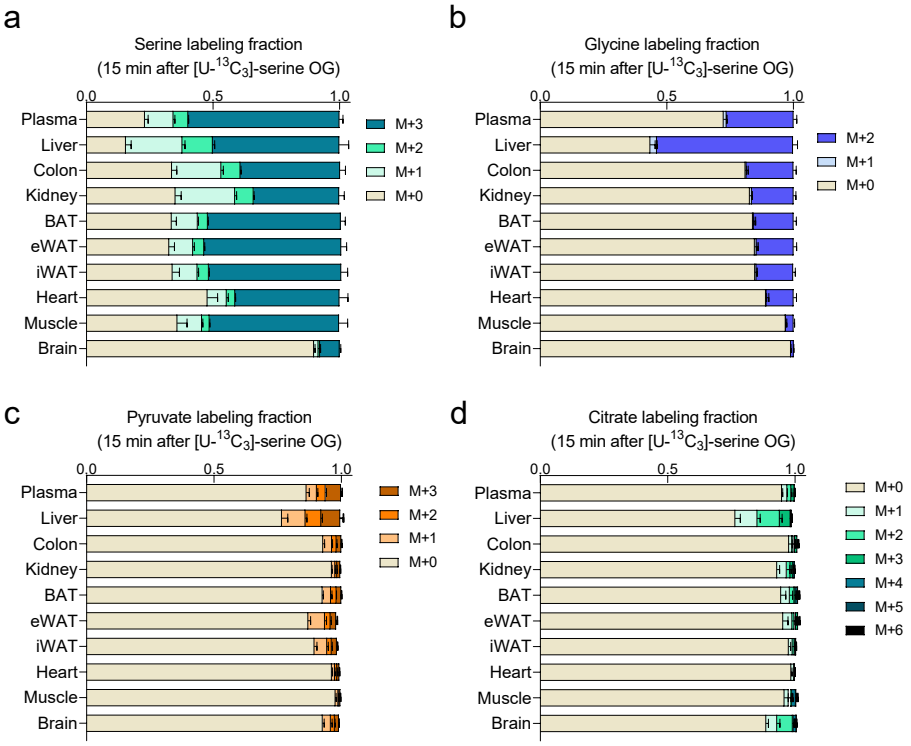

Figure 7. Liver is central to serine metabolism *in vivo*. Tissue serine (a), glycine (b), pyruvate (c), and citrate (d) labeling 15 minutes after [U-^{13}C]serine administration via oral gavage in C57BL6 mice at a dose of 400 mg/kg. Data are mean ± SEM.

SUMMARY

The major accomplishment of this project is establishment of stable isotope tracer infusion method in the lab and quantification of whole-body glucose, serine, and glycine turnover fluxes in health and disease. Consequently, we

demonstrate that whole-body rates of glucose flux are increased in *db/db* mice consistent with earlier clinical studies. On the other hand, we reveal that serine and glycine turnover fluxes are not affected by obesity and diabetes. Moreover, we developed STT that allows for quantitative determination of serine pharmacokinetics. Using [U-^{13}C]serine demonstrate that serine is predominantly metabolized in the mammalian liver into glucose and glycine, and finally using STT we show that *db/db* mice have accelerated serine disposal compared to WT mice.

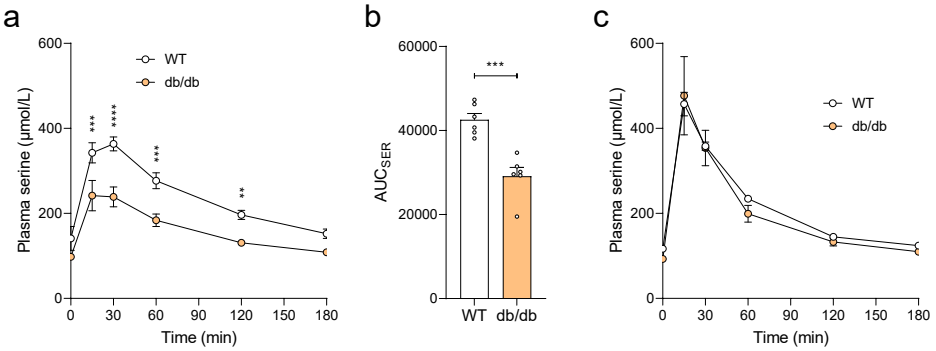

Figure 8. Serine tolerance test revealed differences in whole-body serine disposal in obesity and diabetes. Plasma serine pharmacokinetics in WT and db/db mice administered serine (400 mg/kg) and glucose (2 g/kg; a), area under curve for serine during OGTT/STT (b), and plasma serine pharmacokinetics in WT and db/db mice administered serine (400 mg/kg) only (c). Data are mean ± SEM and were tested using an independent t-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Given COVID impact and NIH NCE, further studies will continue to explore the role of serine metabolism in the development of obesity and *de novo* lipogenesis.

3. Publications

Michal K. Handzlik, Jivani M. Gengatharan, Katie E. Frizzi, Grace H. McGregor, Cameron Martino, Gibraan Rahman, Antonio Gonzalez, Ana M. Moreno, Courtney R. Green, Terry Lin, Yoichiro Ideguchi, Regis J. Fallon, Amandine Chaix, Martina Wallace, Prashant Mali, Rob Knight, Marin L. Gantner, Nigel A. Calcutt, and Christian M. Metallo (2022). Hepatic serine and lipid metabolism drive diabetic peripheral neuropathy. *In revision*.

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