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American Journal of Physiology. Endocrinology and Metabolism

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https://doi.org/10.1152/ajpendo.00163.2022

AMERICAN JOURNAL OF PHYSIOLOGY
ENDOCRINOLOGY AND METABOLISM

Am J Physiol Endocrinol Metab 324: E209–E216, 2023.
First published January 25, 2023; doi:10.1152/ajpendo.00163.2022

RESEARCH ARTICLE

Translational Physiology

Impact of tyrosine kinase inhibitors on glucose control and insulin regulation in patients with chronic myeloid leukemia

Lando Janssen,[1,2] Maria T. E. Hopman,[2] Greetje J. A. Swaans,[2] Neeltje A. E. Allard,[2] Marti Boss,[3] Daphne Lobeek,[3] Martin Gotthardt,[3] Tom J. J. Schirris,[4] Nicole M. A. Blijlevens,[1] and Silvie Timmers[5]

[1]Radboud Institute for Health Sciences, Department of Hematology, Radboud University Medical Center, Nijmegen, The Netherlands; [2]Radboud Institute for Health Sciences, Department of Physiology, Radboud University Medical Center, Nijmegen, The Netherlands; [3]Radboud Institute for Health Sciences, Department of Medical Imaging, Nuclear Medicine, Radboud University Medical Center, Nijmegen, The Netherlands; [4]Radboud Institute for Molecular Life Sciences, Department of Pharmacology and Toxicology, Radboud Centre for Mitochondrial Medicine, Radboud University Medical Center, Nijmegen, The Netherlands; and [5]Human and Animal Physiology, Wageningen University, Wageningen, The Netherlands

Abstract

Treatment with tyrosine kinase inhibitors (TKIs), especially nilotinib, often results in hyperglycemia, which may further increase cardiovascular disease risk in patients with chronic myeloid leukemia (CML). The mechanism underlying the TKI-induced glucose dysregulation is not clear. TKIs are suggested to affect insulin secretion but also insulin sensitivity of peripheral tissue has been proposed to play a role in the pathogenesis of TKI-induced hyperglycemia. Here, we aimed to assess whether skeletal muscle glucose uptake and insulin responses are altered in nondiabetic patients with CML receiving TKI treatment. After a glycogen-depleted exercise bout, an intravenous glucose bolus (0.3 g/kg body weight) was administered to monitor 2-h glucose tolerance and insulin response in 14 patients with CML receiving nilotinib, 14 patients with CML receiving imatinib, and 14 non-CML age- and gender-matched controls. A dynamic [^{18}F]-FDG PET scan during a hyperinsulinemic-euglycemic clamp was performed in a subgroup of 12 male patients with CML to assess m. quadriceps glucose uptake. We showed that patients with CML treated with nilotinib have an increased insulin response to intravenous glucose administration after muscle glycogen-depleted exercise. Despite the increased insulin response to glucose administration in patients with CML receiving nilotinib, glucose disappearance rates were significantly slower in nilotinib-treated patients when compared with controls in the first 15 min after glucose administration. Although [^{18}F]-FDG uptake in m. quadriceps was not different, patients receiving nilotinib showed a trend toward decreased glucose infusion rates during euglycemic clamping when compared with patients receiving imatinib. Together, these findings indicate disturbed skeletal muscle glucose handling in patients with CML receiving nilotinib therapy.

NEW & NOTEWORTHY In this study, we have shown that non-diabetic patients with CML receiving nilotinib therapy show early signs of disturbed skeletal muscle glucose handling, which was not observed in imatinib-treated patients. These observations in nilotinib users may reflect decreased muscle insulin sensitivity, which could serve as a potential target to counteract glycemic dysregulation, and is of clinical importance since these patients have an increased cardiovascular disease risk.

chronic myeloid leukemia; glucose metabolism; insulin sensitivity; skeletal muscle; tyrosine kinase inhibitors

INTRODUCTION

Tyrosine kinase inhibitors (TKIs) are effective first-line therapeutic drugs for patients with chronic myeloid leukemia (CML) (1). To obtain and remain in a major molecular remission, the vast majority of these patients require lifelong TKI therapy (2). After the introduction of imatinib two decades ago, second-generation TKIs such as nilotinib were developed with greater potency and selectivity for the therapeutic target (i.e., BCR-ABL) (3). However, elevated glucose levels are frequently reported in patients with CML treated with nilotinib and may even underlie treatment discontinuation in some patients (4). In fact, grade 3/4 glucose elevations are observed in 7.2% of the nilotinib users after 5 years (5), increasing to up to 8.6% after 10 years (6). Signs of early glycemic dysregulation are already present within the first 3 mo after initiation of nilotinib treatment, and comprise ~75% of all patients with CML at this stage (4). In addition to the glycemic dysregulation, other important metabolic pathways seem to be impaired as suggested by deregulated lipid

Correspondence: L. Janssen (Lando.Janssen@radboudumc.nl); S. Timmers (Silvie.Timmers@wur.nl).
Submitted 12 July 2022 / Revised 5 December 2022 / Accepted 17 January 2023

profiles in these patients (7). This disturbed metabolic state may potentially deteriorate cardiovascular health in nilotinib users (8), since these patients already have an increased risk of cardiovascular events (9, 10).

Alterations in glucose metabolism are not specific to nilotinib therapy, but also occur in patients treated with other TKIs (11). Interestingly, both positive and negative effects on glucose metabolism have been attributed to imatinib (4, 12, 13). Diabetic patients with CML treated with imatinib showed improved glycemic control with a reduction of antidiabetic drug regimens (14). The mechanism underlying these TKI-induced metabolic alterations is not yet fully understood and requires further investigation. A recent study demonstrated a fast development of insulin resistance and compensatory hyperinsulinemia as a possible mechanism of impaired glucose metabolism in patients with CML starting nilotinib treatment (4). In type 2 diabetes, skeletal muscle is considered the primary site where insulin resistance develops, even long before β-cell failure or overt hyperglycemia occurs (15). Therefore, this study was set out to explore whether skeletal muscle glucose handling is disturbed in nondiabetic patients with CML treated with nilotinib or imatinib compared with non-CML controls. Specifically, a glycogen-depleting exercise test was performed to prime skeletal muscle for glucose uptake (16), which was followed by an intravenous glucose bolus. Plasma glucose and insulin responses were monitored over 120 min post glucose injection.

METHODS

Study Population

Chronic phase patients with CML aged ≥ 18 yr and treated with either nilotinib or imatinib for at least 6 mo were recruited from the Department of Hematology at the Radboud University Medical Center (Nijmegen, The Netherlands) and through CMyLife (17), a Dutch online platform for patients with CML. Non-CML controls were recruited through advertisements in local newspapers and at general practitioners' offices. Each patient with CML treated with nilotinib was matched to a patient with CML receiving imatinib treatment and a non-CML control based on gender and age. Subjects with diabetes mellitus, contraindications to exercise testing according to the American College of Cardiology (ACC) and American Heart Association (AHA) guidelines (18), or known allergy to a component of the standardized meal were not eligible. Written informed consent was obtained from all subjects before participation, as approved by the Local Committee on Research Involving Human Subjects of the region Arnhem and Nijmegen, The Netherlands. The study was registered at The Netherlands Trial Registry (NL8176).

Study Procedure

Participants were invited to the research facility for a medical screening, including medical history and standard physical examination. Fasting blood glucose levels were measured to screen for undiagnosed diabetes mellitus (≥7.0 mmol/L). Body composition was determined using single-frequency bioimpedance analysis (Bodystat 1500, Bodystat Ltd., Douglas, Isle of Man, UK). The Short Questionnaire to Assess Health-Enhancing Physical Activity (SQUASH) (19) was used to determine physical activity levels. CML characteristics were obtained from electronic medical records. After inclusion, all participants underwent cardiopulmonary exercise testing with continuous ECG monitoring according to standard laboratory techniques (Lode Excalibur; Groningen, The Netherlands) to determine peak oxygen uptake ($\dot{V}O_{2peak}$) and maximal heart rate (20).

All participants visited the research facility on a separate occasion after an overnight fast for a high-dose intravenous glucose tolerance test after an exercise bout to determine plasma glucose disappearance and insulin response. Participants were instructed to abstain from strenuous exercise for 24 h before testing. Upon arrival, an intravenous cannula was placed into the antecubital vein of each forearm for administration of the glucose bolus and blood withdrawal, respectively. Subsequently, participants received a low-carbohydrate breakfast consisting of Greek yogurt and pecans containing 334 kcal and 5.3 g of carbohydrates. After a resting period of 30 min, participants cycled for 1 h on an ergometer under the supervision of a research assistant, maintaining a constant heart rate (resting heart rate + 70% of heart rate reserve as determined during the incremental cycling test) to deplete skeletal muscle glycogen stores. Directly after exercise, participants were instructed to lie down in supine position, after which an intravenous bolus of 20% (wt/vol) glucose (0.3 g/kg body weight; Baxter B.V., Deerfield, IL) was administered 5 min post exercise over a period of 3 min. Since infusion of glucose after exercise results in a rapid rebuilding of depleted muscle glycogen stores (16), this test allows to detect differences in skeletal muscle glucose uptake between the three groups. Glucose and insulin levels were determined in plasma from blood samples drawn before the start of exercise, at exercise completion, and at 1, 3, 5, 10, 15, 20, 30, 45, 90, and 120 min after glucose infusion. In addition, lactate levels were measured at the completion of exercise. Glucose and lactate levels were determined using a Biosen C-Line glucose analyzer (EKF Diagnostics, Cardiff, UK). For the determination of insulin levels, samples were frozen at the time of collection and assessed by an in-house chemiluminescent immunoassay in batches of ~110 samples within five consecutive days.

A dynamic [^{18}F]-FDG PET scan was performed on a third study day in a subgroup of 12 male participants (n = 6 CML + nilotinib, n = 6 CML + imatinib) to assess skeletal muscle glucose uptake under nonexercise conditions. Participants (mean age: 51 ± 12 yr) were instructed to abstain from smoking, alcohol, caffeine, and strenuous exercise 24 h before testing. To maximize the uptake of the radiopharmaceutical [^{18}F]-FDG in muscle tissue, a hyperinsulinemic (60 mU/m^2/min)-euglycemic (5.0 mmol/L) clamp was started before the PET scan and maintained during the 1-h dynamic scanning period. To this end, an intravenous cannula was inserted into the antecubital vein of the right forearm for infusion of glucose 20% (wt/vol) (Baxter B.V., Deerfield, IL) and insulin (insulin aspart; Novo Nordisk, Bagsvaerd, Denmark). Glucose levels were measured every 5 min in arterialized venous blood obtained from another intravenous cannula inserted into a distal vein of the contralateral forearm, which was positioned in a heated box (55°C). After 30 min of euglycemia, a low-dose CT of the upper leg was acquired (40 mAs and 130 kV) for PET attenuation correction

and anatomic reference. Images were reconstructed with a B31f convolution kernel and slice thickness of 5 mm. Subsequently, 1.6 MBq/kg [^{18}F]-FDG was infused and PET imaging was performed using a Siemens Biograph mCT-40 time-of-flight PET/CT scanner. The PET scan of one patient in the imatinib group was terminated prematurely after 30 min because of severe discomfort. PET/CT images were reconstructed in 33 frames (1×10 s, 8×5 s, 4×10 s, 2×15 s, 6×30 s, 5×120 s, 5×300 s, and 2×600 s) to obtain time-activity curves using PMOD v. 3.15 (PMOD Technologies Ltd., Zürich, Switzerland). Volumes of interest (VOIs) were manually drawn (1.5 mL) in the m. quadriceps of both legs. Tracer activity within the VOIs was expressed as mean standard uptake values (SUV_{mean}), defined as activity per mL of tissue divided by the injected dose in MBq per gram bodyweight.

Statistical Analysis

Continuous data are reported as means ± SD or median with 25th and 75th quartiles and categorical variables as counts and percentages. One-way ANOVA and Kruskal–Wallis with Bonferroni post hoc tests were used for comparison between groups for normally distributed and skewed data, respectively. Fisher's exact test was performed for group comparison of categorical variables. Glucose disappearance rates and insulin responses after exercise were assessed by linear mixed models after log-transformation of the glucose and insulin concentrations post glucose administration. All data analysis was performed using commercially available software (SPSS 22.0, IBM, Armonk, NY). Statistical significance was set at P value <0.05.

RESULTS

A total of 14 patients with CML receiving nilotinib treatment (57% males, mean age 54 ± 11 yr) were matched in a 1:1:1 ratio for gender and age to 14 patients with CML receiving imatinib treatment and 14 non-CML controls. One patient with CML in the imatinib group withdrew from the study after the first study day because she switched to dasatinib treatment due to general feeling of discomfort. The remaining 41 participants completed the entire study protocol. Body composition measures as well as disease characteristics were not different between the groups (Table 1). $\dot{V}O_{2peak}$ was significantly different between the groups ($P = 0.04$); however, pairwise comparison between the groups with Bonferroni post hoc testing did not reach statistical significance ($P_{nilotinib\ vs.\ control} = 0.12$, $P_{nilotinib\ vs.\ imatinib} = 1.0$, $P_{imatinib\ vs.\ control} = 0.07$). Fasting glucose and insulin levels were not different between the groups (both $P > 0.05$; Fig. 1). The median Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index was 2.1 (interquartile range, IQR 1.6–3.8), 1.6 (IQR 1.2–2.5), and 1.5 (IQR 1.1–2.2) in the nilotinib, imatinib, and control group, respectively ($P = 0.16$). In addition, post-prandial glucose levels were not different between the groups ($P = 0.61$; Fig. 1).

Effects of TKIs on Glucose Handling and Insulin Action

Plasma glucose and insulin levels were determined after completion of the exercise protocol and over a period of 120 min after intravenous glucose administration (Fig. 2). Directly after 1 h of cycling exercise, plasma glucose levels did not differ between patients with CML receiving nilotinib

Table 1. *Subject characteristics*

Characteristics	Total	CML + Nilotinib	CML + Imatinib	Controls	*P* Value
Subject number, *n*	42	14	14	14	
Gender, male:female	24:18	8:6	8:6	8:6	1.00
Age, years	53 ± 11	54 ± 11	53 ± 11	54 ± 12	0.96
Body composition					
Weight, kg	80.3 ± 14.8	84.2 ± 19.9	82.2 ± 9.2	74.7 ± 12.6	0.21
BMI, kg/m^2	26.0 ± 3.6	26.5 ± 4.6	26.7 ± 3.2	24.8 ± 2.8	0.35
Waist to hip ratio	0.93 ± 0.07	0.94 ± 0.10	0.94 ± 0.05	0.90 ± 0.06	0.22
FFMI*, kg/m^2	19.2 ± 2.6	19.2 ± 3.1	19.3 ± 1.7	19.1 ± 2.9	0.98
FMI, kg/m^2	6.8 ± 2.9	7.3 ± 3.4	7.4 ± 3.1	5.7 ± 1.9	0.26
Physical fitness					
Physical activity, METmin/week	2,930 (1,700–4,269)	3,107 (1,523–4,502)	2,415 (1,692–4,312)	3,527 (2,380–4,495)	0.53
$\dot{V}O_{2peak}$, mL/min	2,678 ± 775	2,688 ± 723	2,541 ± 786	2,803 ± 848	0.68
$\dot{V}O_{2peak}$, mL/min/kg	33.4 ± 7.4	31.8 ± 4.1	31.1 ± 7.9	37.4 ± 8.1	0.04†
Max heart rate, bpm	172 ± 13	172 ± 12	174 ± 15	169 ± 12	0.55
Max workload, watt	231 ± 66	230 ± 64	219 ± 71	243 ± 65	0.63
CML characteristics					
Daily TKI dose					
150 mg, N	1	1			
300 mg, N	3	3			
400 mg, N	12		12		
600 mg, N	11	9	2		
800 mg, N	1	1	-		
Time since Dx, months	59 (22–146)	61 (20–136)	53 (27–157)		0.77
Duration of current TKI therapy, mo	49 (19–72)	32 (13–69)	49 (28–157)		0.16
CML activity, BCR-ABL^{IS} transcript level					
MMR or deeper, N	25 (89%)	12 (86%)	13 (93%)		0.50

Data are presented as means ± SD, percentages or median (interquartile range). CML, chronic myeloid leukemia; BMI, body mass index; FFMI, fat-free mass index; FMI, fat mass index; MET, metabolic equivalent of task; bpm, beats per minute; Dx, diagnosis; TKI, tyrosine kinase inhibitor; IS, international scale; MMR, major molecular response. *Calculated by the equation of Kyle et al. (33). †Bonferroni post hoc testing did not show significant differences between the groups ($P_{nilotinib\ vs.\ control} = 0.12$, $P_{nilotinib\ vs.\ imatinib} = 1.0$, $P_{imatinib\ vs.\ control} = 0.07$).

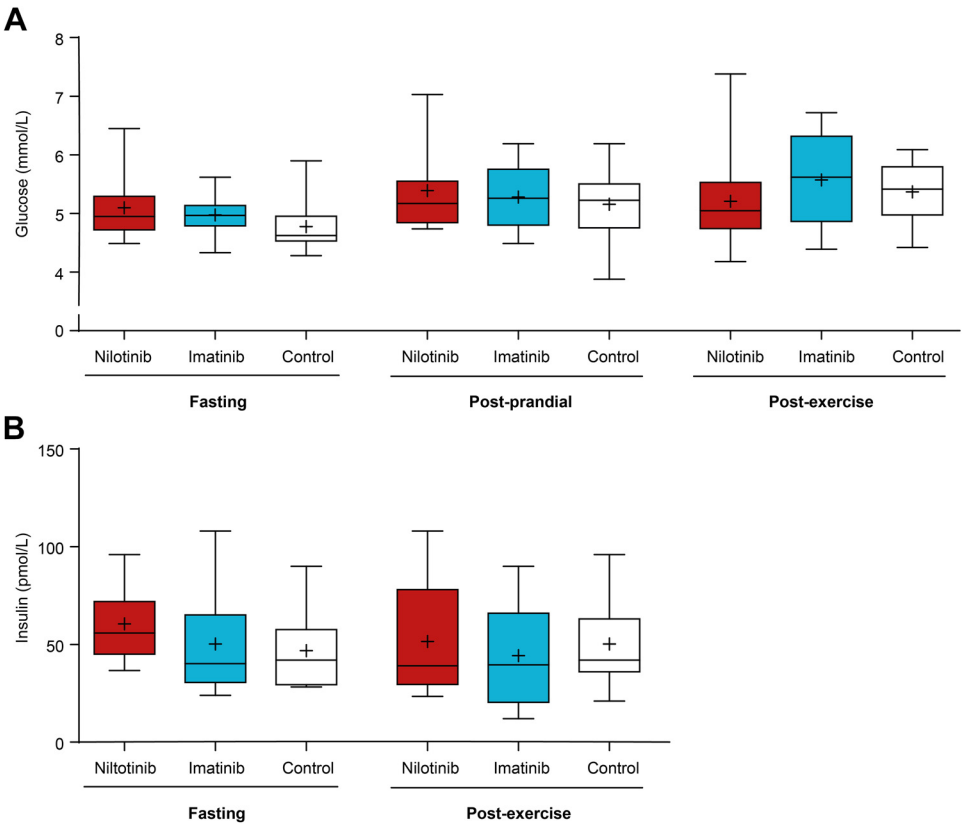

Figure 1. Plasma glucose (*A*) and insulin (*B*) levels in fasting, post-prandial, and post-exercise conditions (i.e. directly at exercise completion). Boxes represent interquartile (25th to 75th percentile) ranges, whiskers represent 5th to 94th percentile, and the horizontal line and cross indicate the median and mean, respectively. n = 14 per group in patients receiving nilotinib therapy and controls, n = 13 in the imatinib group.

or imatinib treatment and non-CML controls [5.0 (IQR 4.7–5.5 mmol/L) vs. 5.6 (IQR 4.8–6.3 mmol/L) vs. 5.4 (IQR 5.0–5.8 mmol/L), respectively; P = 0.33], neither did insulin levels [39.0 (IQR 29.6–78.0 pmol/L) vs. 39.6 (IQR 20.4–66.0 pmol/L) vs. 42.0 (IQR 36.0–63.0 pmol/L), respectively; P = 0.60; Fig. 1] nor lactate levels [1.9 (IQR 1.6–2.5 mmol/L) vs. 1.9 (IQR 1.7–2.6 mmol/L) vs. 1.6 (IQR 1.3–2.1 mmol/L), respectively; P = 0.26]. Plasma glucose levels after administration of the glucose bolus were not different between the groups ($P_{\text{nilotinib vs. control}}$ = 0.32, $P_{\text{nilotinib vs. imatinib}}$ = 0.67, $P_{\text{imatinib vs. control}}$ = 0.59). The plasma glucose disappearance rate was slower in patients with CML receiving nilotinib treatment compared with controls in the first 15 min ($P_{\text{Time×(nilotinib vs. control)}}$ = 0.037, $P_{\text{Time×(nilotinib vs. imatinib)}}$ = 0.70, $P_{\text{Time×(imatinib vs. control)}}$ = 0.09), but did not differ over the total period of 120 min following glucose administration ($P_{\text{Time×(nilotinib vs. control)}}$=0.71, $P_{\text{Time×(nilotinib vs. imatinib)}}$ = 0.81, $P_{\text{Time×(imatinib vs. control)}}$ = 0.90; Fig. 2*A*). Plasma insulin levels after glucose administration were higher in nilotinib-treated patients compared with both non-CML controls ($P_{\text{nilotinib vs. control}}$ = 0.001) and imatinib-treated patients with CML ($P_{\text{nilotinib vs. imatinib}}$ = 0.027), but did not differ between imatinib-treated patients with CML and non-CML controls ($P_{\text{imatinib vs. control}}$ = 0.30; Fig. 2*B*). The decrease in insulin levels was not different between groups ($P_{\text{Time×(nilotinib vs. control)}}$ = 0.47, $P_{\text{Time×(nilotinib vs. imatinib)}}$ = 0.59, $P_{\text{Time×(imatinib vs. control)}}$ = 0.86; Fig. 2*B*). Linear regression analysis showed a higher increase in plasma insulin levels per unit of glucose for nilotinib-treated patients with CML [48.0 (95%CI: 41.3–54.7 pmol/L)] compared with patients with CML receiving imatinib [36.9 (95%-I: 29.2–44.6 pmol/L; P = 0.029)] and controls [37.2 (95%CI: 30.5–43.9 pmol/L); P = 0.029; Fig. 2*C*].

Skeletal Muscle Glucose Uptake under Hyperinsulinemic Conditions Assessed by Dynamic [^{18}F]-FDG PET

Six nilotinib-treated and six age-matched imatinib-treated patients with CML underwent a [^{18}F]-FDG PET scan for the assessment of skeletal muscle glucose uptake under hyperinsulinemic conditions (mean age: 51 ± 12 yr). The glucose infusion rates (GIRs) needed to maintain euglycemia (Fig. 3*A*) were numerically lower in patients with CML receiving nilotinib [6.8 ± 1.8 mg kg fat-free mass (FFM)$^{-1}$ min^{-1}] compared with patients receiving imatinib (9.4 ± 2.8 mg kg FFM^{-1} min^{-1}), but this difference did not reach statistical significance (P = 0.07; Fig. 3*B*). The dynamic [^{18}F]-FDG PET-scan of one patient in the imatinib group was terminated prematurely after 30 min because of severe discomfort. Quantification of the uptake of [^{18}F]-FDG in the right (Fig. 4*A*) and left (Fig. 4*B*) m. quadriceps during the dynamic PET-CT scan did not result in a significant difference among groups after 60 min (P = 0.53 and P = 0.30, respectively). Representative dynamic PET-CT cross-sectional images of participants with low and high [^{18}F]-FDG uptake in upper leg muscles are shown in Fig. 4*C*.

DISCUSSION

This study demonstrated that patients with CML receiving nilotinib therapy show disturbances in skeletal muscle glucose handling as indicated by *1*) the increased insulin response to control skeletal muscle glucose uptake after muscle glycogen depletion exercise compared with patients receiving imatinib treatment and non-CML controls and *2*) the slower glucose

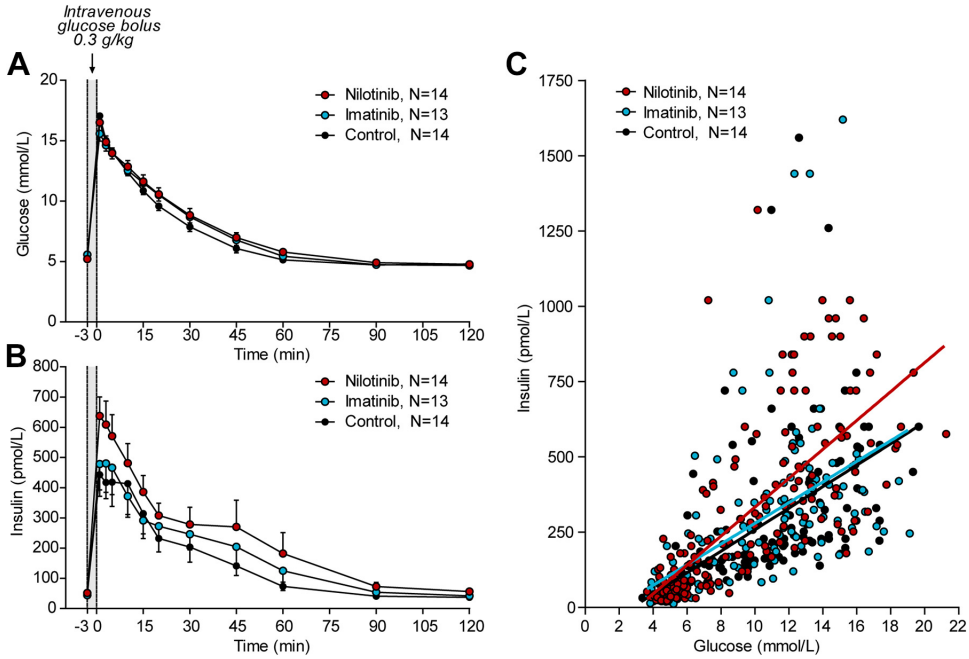

Figure 2. Plasma glucose (*A*) and insulin levels (*B*) after an intravenous glucose bolus (0.3 g/kg body weight) after 1 h of cycling exercise in patients with chronic myeloid leukemia (CML) receiving nilotinib treatment (n = 14), patients with CML receiving imatinib (n = 13) treatment and non-CML controls (n = 14). Error bars represent SEMs. Glucose-to-insulin ratios in nilotinib-treated patients with CML, imatinib-treated patients with CML, and non-CML controls (*C*). Solid lines show linear regression.

disappearance rate in the first 15 min following intravenous glucose administration when compared with controls. Despite normal fasting glucose and insulin levels, these findings may reflect early signs of a decreased insulin sensitivity, and stress the clinical importance to carefully monitor glucose and insulin responses, beyond the fasting state, to prevent further glucometabolic deterioration in patients with CML receiving nilotinib therapy. Furthermore, it highlights the importance of proper patient selection for nilotinib therapy.

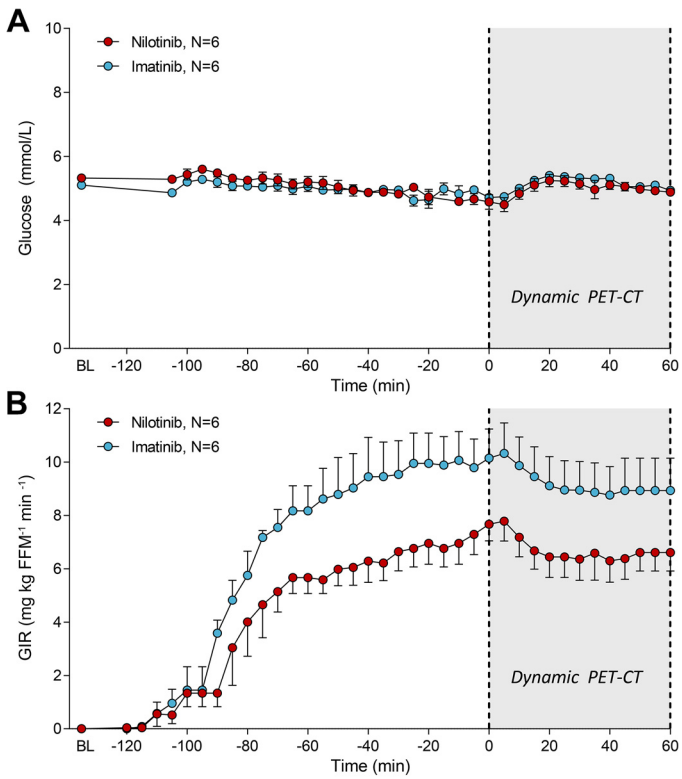

Figure 3. Mean plasma glucose (*A*) and glucose infusion rates (GIR; *B*) during a hyperinsulinemic-euglycemic clamp in a subgroup of patients with chronic myeloid leukemia receiving nilotinib (n = 6) and imatinib therapy (n = 6). Values are shown before and during the dynamic [^{18}F]-FDG PET. Error bars represent SEMs.

Although fasting glucose and insulin levels were not different between groups, differences in glucose regulation became apparent in nilotinib-treated patients with CML, compared with imatinib and non-CML controls when specific skeletal muscle glucose uptake was stimulated. That is, the glucose bolus after the exercise bout resulted in significantly higher plasma insulin levels in patients receiving nilotinib therapy compared with both imatinib-treated patients with CML and non-CML controls. This was coupled to slower glucose disappearance rates during the first 15 min following glucose administration in nilotinib users when compared with controls. These findings are supported by our clinical observations in which we have found a tendency toward lower skeletal muscle glucose uptake as evidenced by a lower glucose infusion rate (GIR) during steady-state hyperinsulinemic-euglycemic clamp conditions in patients with CML receiving nilotinib compared with patients receiving imatinib treatment. However, quantification of skeletal muscle glucose uptake, by administration of a [^{18}F]-FDG bolus during the steady state of the hyperinsulinemic-euglycemic clamp, did not result in a significant difference. The fact that this difference did not reach statistical significance can probably be explained by the explorative nature and hence small sample size and the observed interindividual variability in the subgroup. In addition, other insulin-sensitive tissues such as adipose tissue and liver may be affected by TKI treatment. However, it should be noted that muscle tissue accounts for ~80% of the glucose disposal under euglycemic hyperinsulinemic conditions (15). Elevated lactate levels are also linked to insulin resistance, by suppressing glycolysis and impairing insulin signaling (21). However, lactate levels after the submaximal exercise bout were comparable among groups. Yet, the effects of nilotinib therapy on insulin

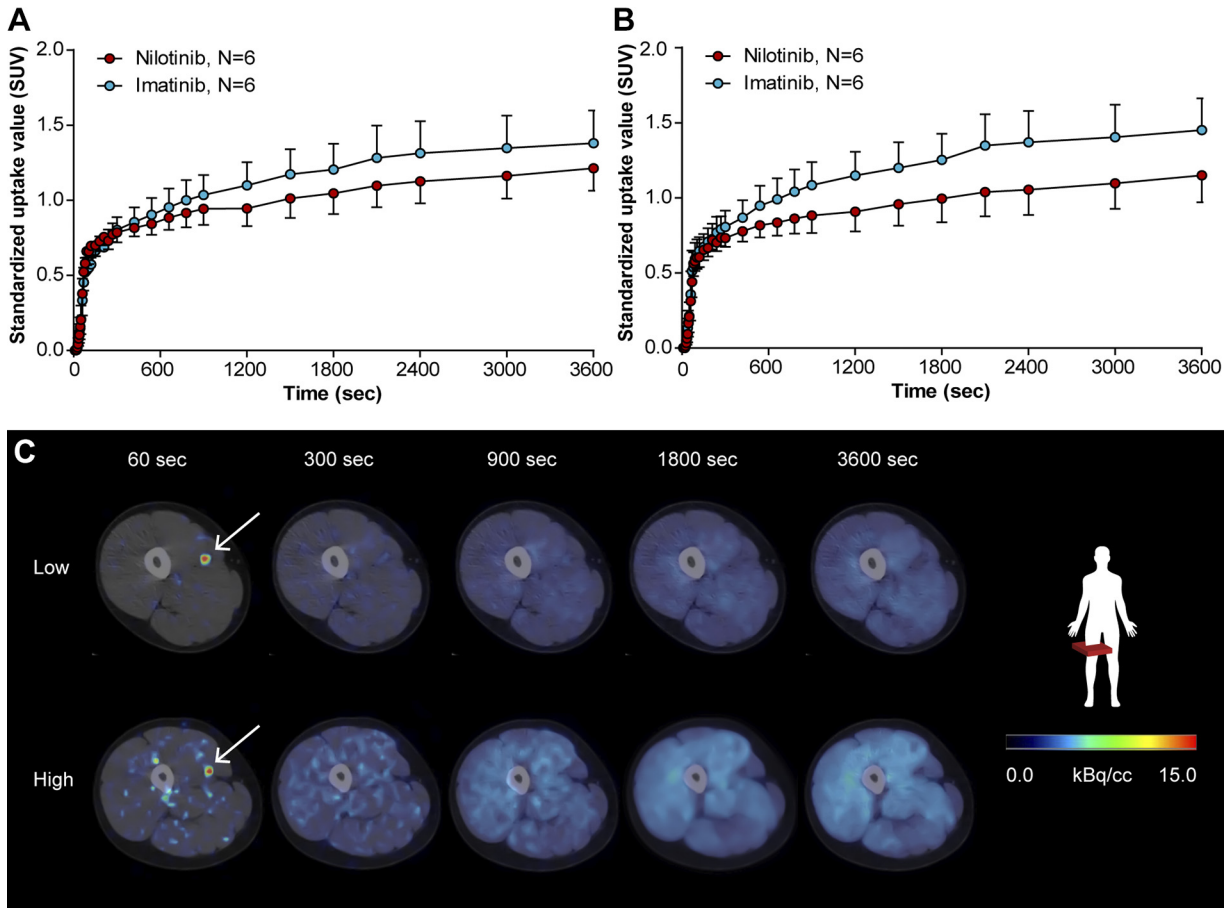

Figure 4. [^{18}F]-FDG uptake expressed as mean standard uptake values during dynamic PET scanning under euglycemic hyperinsulinemic conditions in right (*A*) and left (*B*) m. quadriceps. Error bars represent SEMs. n = 6 per group. Axial PET/CT images of participants with low (upper series) and high (lower series) [^{18}F]-FDG uptake in muscles of the upper leg during dynamic PET scanning at 60, 300, 900, 1,800, and 3,600 s (*C*). The standardized uptake values (SUV) of [^{18}F]-FDG accumulation are presented as cumulative data at the end of each frame. White arrows indicate femoral artery.

sensitivity may have been underestimated as patients with diabetes were excluded.

Altogether, these findings are in line with the study of Racil et al. (22) who proposed decreased peripheral insulin sensitivity with compensatory hyperinsulinemia as an underlying mechanism for nilotinib-induced hyperglycemia, and contradict a decrease in endogenous insulin secretion as an underlying mechanism for nilotinib-induced diabetes mellitus (23). Interestingly, the metabolic disturbances associated with nilotinib therapy have also been linked to adipocyte toxicity (4, 22, 24), but this was beyond the scope of our study.

We did not find alterations in glucose regulation in patients with CML treated with imatinib. Both positive and negative effects on glucose regulation have been reported upon initiation with imatinib therapy. Although some studies found an increase in fasting plasma glucose levels in imatinib-treated patients (4, 13), others observed decreased plasma glucose levels compared with pretreatment levels (12). All these studies did not correct for any changes in weight over time, which may have confounded the results. In this respect, it is important to note that there were no differences in BMI and body composition among the age- and gender-matched groups in our study. Recent literature suggests that the effects of imatinib differ between normoglycemic and diabetic patients. That is, initiation of imatinib therapy resulted in significantly higher fasting glucose levels in normoglycemic patients, whereas a significant decrease in fasting glucose levels was observed in diabetic patients (25). The beneficial effects of imatinib on glucose metabolism in diabetic patients may be linked to a direct effect on pancreatic cells, as supported by the finding that imatinib protects against β-cell death in vitro (26). The paradoxical effects on glucose metabolism of imatinib and nilotinib may be explained by differences in the off-target profiles of TKIs (27). In addition, differences in potency against these off-targets (e.g., PDGFR, c-Kit, Abl, DDR) may also play a role. However, more molecular evidence is warranted to substantiate this idea.

Little is known about the long-term effects of the TKI-induced metabolic dysregulation, since TKIs have only been used for two decades for the treatment of patients with CML. The results of our study suggest that nilotinib users are at risk of developing decreased insulin sensitivity, which may further increase the risk of developing cardiovascular diseases (8, 28). Prevention of insulin resistance in young adults would prevent ~42% of myocardial infarctions (29). This is of great clinical importance since the majority of the patients with CML require lifelong TKI treatment and could therefore

profit from prevention strategies aimed at improving the glucometabolic profile. This is supported by the finding that nilotinib-induced elevated glucose levels are often accompanied by changes in BMI (30). Physical activity can counteract dysregulated glucose metabolism in insulin-resistant skeletal muscle (31) and may therefore be advised to nilotinib users.

This study has extensively assessed glucose control and insulin regulation in patients with CML on TKIs. Although we were able to clearly demonstrate an increased insulin response in nilotinib-treated patients with CML, there are also some limitations to our study. Due to the low incidence of CML, we were not able to conduct a study with a longitudinal design starting at treatment initiation. Hence, it is unknown whether there were differences in glucose metabolism between the groups at treatment initiation. However, we were able to match our study groups in a 1:1:1 ratio based on age and gender. In addition, body composition measures as well as physical activity levels did not significantly differ between the groups. This study assumes that skeletal muscle mass, measured with bioimpedance, is equal between groups. Yet, it would have been more accurate to quantify muscle mass with a DEXA scan or computed tomography (CT). Absolute $\dot{V}O_{2peak}$ (mL/min) was similar among groups, but $\dot{V}O_{2peak}$ adjusted for body weight tended to be lower in patients with CML compared with non-CML controls due to a nonsignificantly higher fat mass (and not fat-free mass) in patients with CML. Since maximal aerobic exercise capacity is mainly dependent on fat-free mass (32), we do not believe that there are relevant differences in exercise capacity between the groups which could have influenced the outcomes of our study. Furthermore, the number of patients who underwent a hyperinsulinemic-euglycemic clamp in combination with dynamic PET-CT scanning to measure skeletal muscle-specific glucose uptake, was too low to provide conclusive results. This is due to the invasive, and time- and cost-consuming nature of this imaging technique.

In conclusion, this study suggests that patients with CML receiving nilotinib therapy show early signs of disturbed skeletal muscle glucose handling, despite fasting normoglycemia and insulinemia. The molecular mechanism underlying the effects of nilotinib on skeletal muscle glucose metabolism warrants more investigation; however, physicians should be aware that decreased muscle insulin sensitivity may already be present in nilotinib users with normal fasting glucose and insulin levels. These observations underscore the importance to monitor glucose and insulin responses, beyond the fasting state, to prevent and reverse disturbed skeletal muscle glucose handling in an early stage. Furthermore, our findings may aid in the selection of TKI therapy for the personalized care of patients with CML.

DATA AVAILABILITY

Data will be made available upon reasonable request.

ACKNOWLEDGMENTS

The authors thank Dr. R. Boellaard and Dr. M.M. Yaqub of the Department of Radiology and Nuclear Medicine at the Amsterdam University Medical Center for their assistance with the analysis of the dynamic [^{18}F]-FDG PET images.

GRANTS

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.J., M.T.E.H., M.G., N.M.B., and S.T. conceived and designed research; L.J., G.J.A.S., N.A.E.A., and M.B. performed experiments; L.J., G.J.A.S., M.B., D.L., and M.G. analyzed data; L.J., M.T.E.H., N.A.E.A., M.B., D.L., M.G., T.J.J.S., N.M.A.B., and S.T. interpreted results of experiments; L.J. prepared figures; L.J. drafted manuscript; L.J., M.T.E.H., G.J.A.S., N.A.E.A., M.B., D.L., M.G., T.J.J.S., N.M.A.B., and S.T. edited and revised manuscript; L.J., M.T.E.H., G.J.A.S., N.A.E.A., M.B., D.L., M.G., T.J.J.S., N.M.A.B., and S.T. approved final version of manuscript.

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