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Functional imaging of skeletal muscle glucose metabolism by ^{18}FDG PET to characterize insulin resistance in patients at high risk for coronary artery disease*

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ABSTRACT

Insulin resistance is associated with several coronary risk factors and is thought to play a critical role for the development of coronary artery disease. Insulin resistance has several causes, including an impaired skeletal muscle glucose utilization rate (SMGU), reduced peripheral blood flow, and altered fatty tissue metabolism, with SMGU being considered the most important. Nonetheless, insulin resistance has only been estimated by the glucose disposal rate (GDR) in previous studies. Methods: Skeletal muscle metabolic imaging with ^{18}FDG and positron emission tomography (PET) was undertaken to measure SMGU during hyperinsulinemiceuglycemic clamping in 22 normotensive type-2 diabetics under no medications (T2DM), 17 normotensive non-diabetic hypertriglyceridemics, 22 patients with hypertension, and 12 age-matched controls. Whole body insulin resistance was assessed by the GDR during hyperinsulinemiceuglycemic insulin clamping. Results: The SMGU and GDR were significantly reduced in T2DM (32.1 ± 16.6 μmol/min/kg and 24.3 ± 13.0 μmol/min/kg, respectively), hypertriglyceridemics (36.5 ± 13.5 μmol/min/kg and 22.7 ± 8.07 μmol/min/kg respectively) and patients with hypertension (35.4 ± 26.6 μmol/min/kg and 29.0 ± 9.90 μmol/min/kg, respectively) compared with controls (72.2 ± 44.1 μmol/min/kg and 43.0 ± 22.9 μmol/min/kg, $p < 0.01$, respectively). In all groups studied, SMGU was significantly correlated with GDR ($r = 0.76$, $p < 0.01$) and GDR ($F = 13.9$) was independently related to SMGU ($r = 0.81$, $p < 0.01$). Conclusion: Insulin resistance is significantly associated with SMGU to a similar degree among patients with T2DM, essential hypertension and hypertriglyceridemia. ^{18}FDG PET functional imaging allows insulin resistance to be assessed.

Keywords: Insulin Resistance; Glucose Metabolism; Skeletal Muscle; Coronary Risk Factor; Type II Diabetes Mellitus; Hypertension; Hyperlipidemia;^{18}F-FDG; PET

1. INTRODUCTION

Insulin resistance, which is present in several conditions that carry increased risk of coronary artery disease (CAD), is thought to play a central role in the development of CAD [1-12]. Several factors, including impaired skeletal muscle glucose utilization (SMGU) [2], reduced peripheral muscle blood flow [13], and altered fatty tissue metabolism [14,15], have been shown to contribute to insulin resistance. Among these factors, SMGU is suggested to be the most essential. It remains to be clarified whether SMGU is equally impaired among patients with different coronary risk factors or if in certain patient subgroups peripheral blood flow abnormality makes a larger contribution to whole body insulin resistance than does SMGU. However, the GDR provides only an estimate of the sum of several factors that contribute to insulin resistance. Therefore, it is anticipated that SMGU imaging will provide much more detailed information for characterizing whole body insulin resistance in subjects with several coronary risk factors. Although the presence of insulin resistance has been shown in association with several different coronary risk factors, including essential hypertension, Type-2 diabetes mellitus (T2DM), hypertriglyceridemia, and cigarette smoking [1-12], a direct diagnostic approach to perform clinical comparative non-invasive studies of various aspects of insulin resistance among patients coronary risk factors.

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Recently, positron emission tomography (PET) has emerged as a technique to allow *in vivo* quantitative analysis of tissue metabolism. This has made it possible to quantify glucose metabolism in skeletal muscle [16-19] by ^{18}FDG PET. As a result, reduced SMGU in patients with T2DM [19] and in patients with mild hypertension [18] has been documented. However, it has not been clarified whether whole body insulin resistance and SMGU, or the relationship between SMGU and GDR, differ among subjects at high risk for CAD. Furthermore, it has not been investigated whether the static skeletal muscle ^{18}F-FDG uptake images differ according to the coronary risk factor present. Thus, the current study was undertaken using the ^{18}F-FDG PET method to compare SMGU values and also the relationship between SMGU and whole body insulin resistance among groups of subjects at high risk for CAD.

1.1. Materials and Methods

1.1.1. Patient Population

We studied 22 T2DM patients without hypertension on dietary therapy, 22 non-diabetic, non-hypertriglyceridemic patients with essential hypertension (HTN), 17 non-diabetic hypertriglyceridemics without hypertension (HTG) and 12 asymptomatic age-matched healthy controls (mean age 48.5 ± 9.88 yr; 10 males, 2 females). None of the patients was being medicated. Patients with typical angina or significant CAD were excluded. The general characteristics of the study subjects are shown in **Table 1**. Before beginning, we informed all subjects of the nature of the study, after which they agreed to participate in the study protocol, which had been approved by the local Ethics Committee.

Table 1. General characteristics of the study subjects.

	Controls	T2DM	HTN	HTG
N (M/F)	12 (10/2)	22 (17/5)	22 (16/6)	17(13/4)
Age	48.5 ± 9.88	50.9 ± 8.29	53.4 ± 10.1	46.8 ± 9.35
BW	60.6 ± 11.5	58.6 ± 10.8	65.8 ± 11.0	53.8 ± 9.7
HT	161.0 ± 8.2	161 ± 9.39	162 ± 8.17	158 ± 8.79
BMI	23.3 ± 2.51	23.3 ± 1.95	25.4 ± 3.17	25.3 ± 1.02
HbA1c	5.7 ± 0.30	7.96 ± 1.58*	5.5 ± 0.33	5.25 ± 0.42
FBS	5.21 ± 0.79	8.96 ± 2.41	5.25 ± 0.47	4.90 ± 0.54
TC	4.86 ± 0.63	4.96 ± 0.34	188 ± 27.6	5.48 ± 0.56
HDL	1.44 ± 0.69	1.32 ± 0.30	44.1 ± 10.7	35.2 ± 11.4
TG	1.25 ± 0.31	1.69 ± 1.18	164 ± 121	570 ± 243
LDL	3.11 ± 0.45	2.82 ± 0.51	92.8 ± 49.7	3.50 ± 0.54
SBP(R)	121 ± 6.4	125 ± 11.3	166 ± 24.4	134 ± 20.6
DBP(R)	72.0 ± 6.5	73.3 ± 10.6	90.0 ± 15.1	82.0 ± 13.8
HR(R)	61.3 ± 10.4	66.4 ± 13.1	61.0 ± 7.42	66.4 ± 6.51
FFA	0.35 ± 0.16	1.02 ± .92	0.69 ± .80	1.01 ± .55
Insulin	54.0 ± 29.0	53.3 ± 25.5	63.1 ± 20.8	57.2 ± 12.4

N: number, M/F: male/female, T2DM: Type-2 diabetes mellitus, HTN; hypertension, BW: body weight (kg), HT: height (cm), BMI: body mass index (kg/m^2), BPS: systolic blood pressure (mmHg), BPD: diastolic blood pressure (mmHg), HbA1c: hemoglobin A1c (%), FBS: fasting plasma blood glucose concentration (mmol/liter), TC: total cholesterol (mmol per liter), HDL: high density lipoprotein cholesterol (mmol per liter), TG: triglycerides (mmol per liter), LDL: low density lipoprotein cholesterol (mmol per liter), FFA: free acids concentration (mEq/liter), NS: not significant, *$p < 0.01$ vs T2DM

1.1.2. Positron Emission Tomography (PET)

1.1.2.1. Preparation of ^{18}FDG

^{18}F was produced with a cyclotron Cypris model 370 (Sumitomo JYUKI Industries, LTD., Tokyo, Japan). Synthesis of ^{18}FDG was undertaken using automated system according to the technique developed by Ehrenkaufer *et al*. [20] Radiochemical purity of ^{18}FDG was greater than 95%.

1.1.2.2. Acquisition of Skeletal Muscle Metabolic Images

Skeletal muscle ^{18}FDG images were given using a Headtome IV PET scanner (Shimadzu Corp., Kyoto, Japan). This Headtome IV PET scanner owns 7 imaging planes; in-plane resolution is 4.5 mm at full width at half maximum (FWHM) and the z-axial resolution is 9.5 mm at FWHM. After using a smoothing filter, 7 mm of effective in-plane resolution was given. This Headtome IV PET scannerhasa sensitivity of 14 and 24 kcps (μCi/ml) for direct and cross planes, respectively. All dynamic PET scans were undertaken during insulin clamping which began 2 hours before the injection of ^{18}FDG.

After transmission data was taken to correct photon attenuation, we injected ^{18}FDG (5 - 10 mCi) and collected dynamic PET data for 52 min. During this interval, we obtained 19 and 5 dynamic scans for the thoracic and femoral regions, respectively, using the following protocol: for the thoracic region, nine 10-sec, three 30-sec, three 120-sec, two 240-sec, one 300-sec and one 600-sec scan; for the femoral region, one 120-sec scan and four 300-sec scans were obtained.

1.1.2.3. Quantification of the SMGU

According to the quantitative method which was developed by Ohtake *et al*. [21] and Yokoyama *et al.* [22] input function to determine SMGU was acquired from the time activity curve of the descending aorta corrected by sampling venous blood 7 times during dynamic data acquisition for thoracic regions and 5 times during dynamic data acquisition for thigh muscle over 22 minutes. Using the input function, we determined $k_1 \times k_3/(k_2 + k_3)$ by Patlack graphic analysis and calculated the SMGU by substituting $k_1 \times k_3/(k_2 + k_3)$ in the following equation.

$SMGU = (k_1 \times k_3/(k_2 + k_3)) \times$ (average blood glucose concentration)/Lumped constant (LC)

k_1 and k_2 and k_3 were rate constants of the following ^{18}FDG tracer kinetic three compartment model.

$$\text{Glu (S)} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \text{Glu (skelt. M)} \underset{k_4}{\overset{k_3}{\rightleftarrows}} \text{Glu-6-phosphate (skelt. M)}$$

Glu = glucose, S = serum, skelt. = skeletal, M = muscle and k_4 is assumed to be zero in the skeletal muscle. Lumped constant stands for the difference between ^{18}FDG and glucose, which was calculated to be 1.0 in skeletal muscle cells, as reported in human studies [16, 17].

All data were corrected for dead time effects to reduce error to less than 1%. To avoid the influence of the partial volume effect associated with the objects' size, recovery coefficients obtained from experimental phantom studies in our laboratory were used. The recovery coefficient was 1.0 when the muscle wall thickness was more than 30 mm and was estimated to be 1.0 for SMGU because the diameter of femoral muscle was estimated at more than 30 mm.

We obtained data on the SMGU from transaxial images of the femoral muscle in addition to transaxial dynamic data of 7 planes. The total amount of SMGU was determined by averaging these values.

To calculate SMGU we used the Indy high-speed image processing system (Asahi Kasei Information System Co., Ltd., Tokyo, Japan) with "Dr View" software (Asahi Kasei Information System Co., Ltd., Tokyo, Japan).

Quantitative estimation of whole body insulin resistance was made by obtaining the GDR during hyperinsulinemiceuglycemic clamping (μmol/min/kg) using a previously reported method [23].

1.1.3. Statistical Analysis

Data consisting of 2 parameters were analyzed by the two-tailed Student's t test. Data involving 3 parameters were analyzed by analysis of variance. A value of $p < 0.05$ was considered statistically significant. Values were expressed as mean ± standard deviation. Multivariate stepwise regression analysis was used to examine which factors among plasma free fatty acid concentration, age, GDR, duration of diabetes, plasma fasting glucose concentration, hemoglobin A1c value, systolic blood pressure and gender were independently related to SMGU.

1.2. Result

1.2.1. GDR

The GDR was significantly reduced in patients with T2DM with no medications (24.3 ± 13.0 μmol/min/kg, $p < 0.05$), HTG (22.7 ± 8.07 μmol/min/kg, $p < 0.05$) and HTN (29.0 ± 9.90 μmol/min/kg, $p < 0.05$) compared with controls (43.0 ± 22.9 μmol/min/kg).

1.2.2. SMGU

The SMGU was significantly reduced in patients with T2DM (32.1 ± 16.6 μmol/min/kg, $p < 0.01$), HTG (36.5 ± 13.5 μmol/min/kg, $p < 0.01$) and HTN (35.4 ± 26.6 μmol/min/kg, $p < 0.05$) compared with controls (72.2 ± 44.1 μmol/min/kg). SMGU was significantly correlated with GDR in patients with T2DM with no medications ($r = 0.72$, $p < 0.01$), HTG ($r = 0.80$, $p < 0.01$) and HTN ($r = 0.76$, $p < 0.01$) (**Figure 1**).

Multivariate regression analysis showed that the GDR ($F = 13.9$) was independently related to SMGU ($r = 0.81$, $p < 0.01$) in all patients studied.

1.2.3. Skeletal Muscle ^{18}FDG Images

Typical femoral muscle static ^{18}FDG PET images are shown in **Figure 2**. Femoral muscle ^{18}FDG uptake was apparently reduced in subjects with T2DM with no medications, HTG and HTN compared with controls visually.

1.2.4. Serum Glucose Concentration

During hyperinsulinemiceuglycemic clamping, the average serum glucose concentration in patients with insulin resistance (4.72 ± 0.66 mmol/liter) was the same as that of control subjects (4.79 ± 0.78 mmol/liter).

1.2.5. Serum Insulin Concentration.

Serum insulin concentration following glucose loading using the hyperinsulinemiceuglycemic clamp technique in the study subjects (57.9 ± 36.7 μU/ml) was comparable with that in controls (54 ± 29 μU/ml). Serum insulin concentrations at the beginning and at the end of the study did not differ significantly.

1.2.6. Serum Free Fatty Acid Concentration (FFA)

Serum FFA during hyperinsulinemiceuglycemic clamping in patients with insulin resistance (entire study group: 0.67 ± 0.28 mEg/liter) was significantly higher than that of normal subjects (0.32 ± 0.19 mEg/liter, $p < 0.01$). Plasma FFA concentrations in each patient subgroup were as follows: T2DM on dietary therapy, 1.02 ± 0.96; HTG, 1.01 ± .55; and HTN, 0.69 ± 0.80.

We observed a significant positive correlation between the GDR and SMGU in each of the disease subgroups studied, that is, in patients with T2DM, essential hypertension and hypertriglyceridemia. An apparently reduced SMGU in femoral muscle was also seen in each of those patient subgroups. Therefore, the SMGU is a major contributor to insulin resistance for each of the coronary risk factors studied. Multivariate stepwise regression analyses have shown that femoral muscle SMGU was the critical factor for GDR. Thus, it can be concluded that femoral muscle functional imaging with ^{18}FDG PET is an appropriate method to analyze insulin resistance in the context of various coronary risk factors.

For our present results, scatter plots of GDR versus SMGU have shown that the range of SMGU values

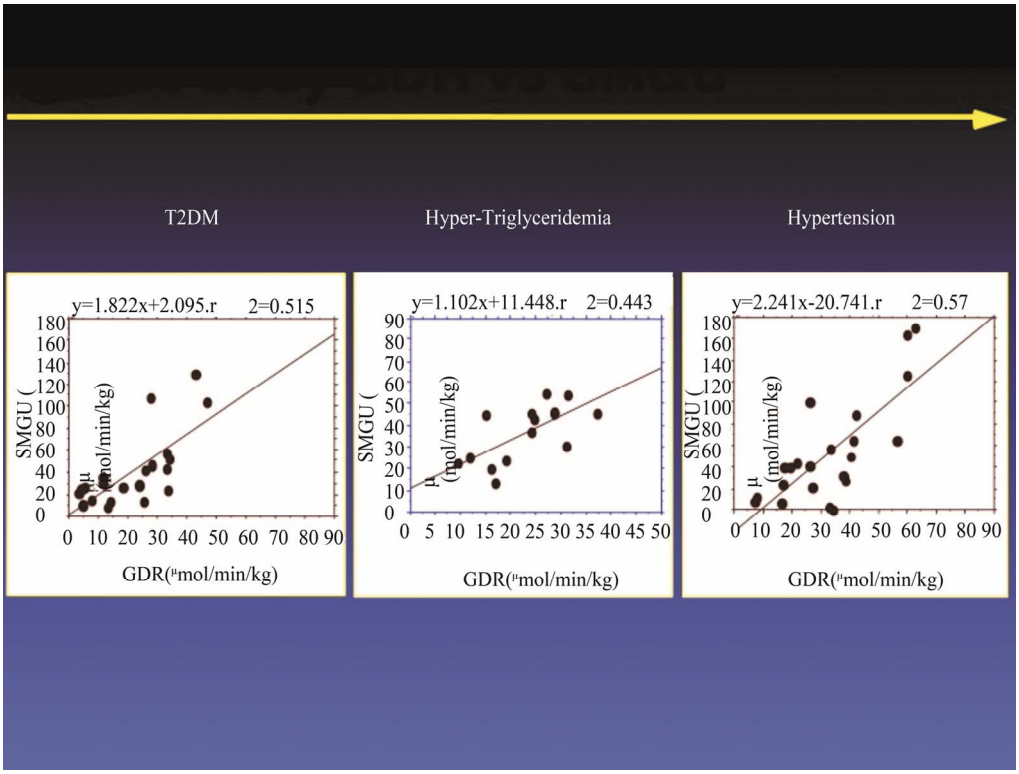

Figure 1. The relationships between skeletal muscle glucose utilization rate (SMGU) and whole body glucose disposal rate (GDR) in each study patients (patients with essential hypertension, hypertriglyc- eridemia and type-2 diabetes mellitus (T2DM)).

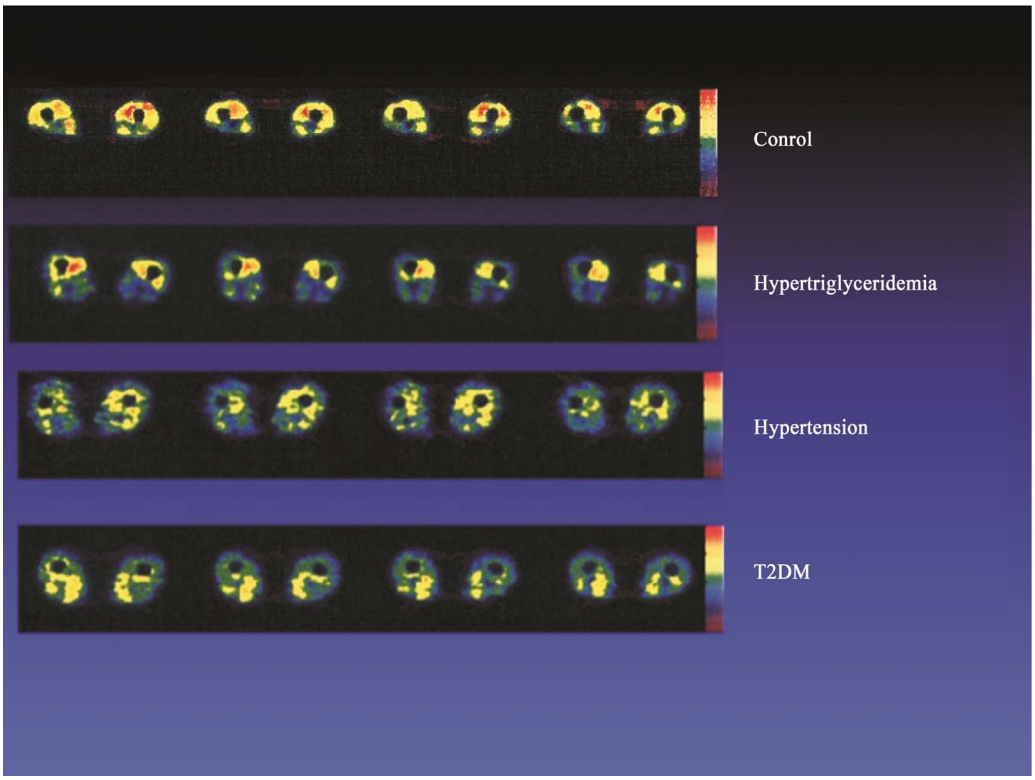

Figure 2. Skeletal Muscle ^{18}F-FDG Images in each study subjects (control, patients with essential hyper- tension, hypertriglyc- eridemia and type-2 diabetes mellitus (T2DM)).

differs among the three groups of patients. A greater range of values for SMGU in HTN and T2DM as compared with HTG has suggested that underlying mechanisms to evoke disease, especially with reference to insulin resistance, differ even in the same group of HTN and T2DM.

It is possible to analyze the influence of factors other than SMGU by noting the difference between whole body glucose utilization rate and SMGU. In general, skeletal muscle weight represents 30% - 40% of total body weight. If skeletal muscle weight is taken as approximately 35% of whole body weight and tissue other than skeletal muscle as 65% of total body weight, this difference was estimated to be reduced in hypertriglyceridemics (15.4 ± 7.67 μmol/min/kg) and T2DM (19.2 ± 10.8 μmol/min/kg) as compared with controls (27.3 ± 16.1 μmol/min/kg) and HTN (25.5 ± 14.1 μmol/min/kg). In contrast, no significant difference was seen between HTN and controls. The results from this study, that reduced SMGU plays essential role on the insulin resistance, is consistent with results of Capaldo *et al*. [24] and Natali *et al*. [25]. Therefore, although the SMGU and GDR were similar among the three groups, the influence of factors other than SMGU on whole body insulin resistance is much greater in HTG and T2DM and less in HTN.

Compared with values in the literature, our SMGU value (72.2 ± 44.1 μmol/min/kg) is somewhat lower than that of Nuutila *et al*. [18], who tested 8 young males and 6 young females (94 ± 9 μmol/min/kg), but somewhat greater than in Laine *et al*. [26] on 7 young males who reported 58 ± 10 μmol/min/kg and Paternostero *et al*. [27] on 6 young males, with 56 ± 6 μmol/min/kg. Overall, our SMGU data on control subjects are similar to averaged data from the literature. Small discrepancies may result from the limited numbers of individuals included in each study, as well as race and gender differences between study populations. In contrast, the GDR in our controls (10 elderly men and 2 elderly females yielding a value of 43.0 ± 22. μmol/min/kg) is similar to data of Nuutila *et al*. [18] where 8 young males and 6 young females yielded 44 ± 3 μmol/min/kg. However, our value is somewhat greater than the values established by Paternostero *et al*. [27] where 6 young males gave a value of 37 ± 4 μmol/min/kg). The SMGU in young patients with HTN was much higher than in our elderly patients with HTN. That may be due to the fact that insulin resistance could progress over time. Differences in life style or race difference could also be factors contributing to this difference.

Because among the various coronary risk factors insulin resistance has been reported to be a common critical factor for the development of CAD [3,28], managing insulin resistance in subjects at high risk for CAD is important. In addition, the effects of therapy on insulin resistance may be much more precisely estimated by functional imaging with ^{18}FDG PET than with GDR alone.

Exercise training is a possible choice of therapy for insulin resistance in patients with or without T2DM. Its beneficial effect on essential hypertension, T2DM and hypertriglyceridemia has been demonstrated, as well as its cost effectiveness. It has been suggested that the skeletal muscle insulin-sensitive glucose transporter (GLUT4) can be experimentally activated by exercise training [29]. Therefore, the effectiveness of exercise training in these disorders supports the hypothesis that improved insulin resistance is achievable via an improvement of SMGU. Several recent investigations have shown that insulin resistance can be improved by medical therapy [30-37]. ^{18}FDG PET would be of use in evaluating the effect of such agents on SMGU and, thus, increase our knowledge of therapeutic strategies for insulin resistance. The present study is the first to test the hypothesis that insulin resistance can be imaged through skeletal muscle functional imaging with ^{18}FDG PET. Future prospective studies should address the issue of whether therapeutic effects on insulin resistance are reflected in the SMGU.

In addition to SMGU, several more minor factors, such as reduced peripheral blood flow and hepatoneogluconenesis, are related to insulin resistance. However, obtaining measurements and information on these factors would be very time consuming (more than 2 days), and for ethical reasons we did not request study patients to undergo these additional tests. This is therefore a limitation of the present study.

2. CONCLUSION

Insulin resistance is highly correlated with SMGU to a similar degree among patients with T2DM, essential hypertension and hypertriglyceridemia. ^{18}FDG PET functional imaging allows insulin resistance to be assessed.

3. AUTHORS' CONTRIBUTIONS

Dr. Yokoyamadesigned the study and recruited study subjects. Drs. Yokoyama, Inoue, Moritanmade PET data sampling and data analysis. Drs. Inoue, Moritan-provided valuable discussion and advice in writing this manuscript.

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