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Plasma dipeptidyl peptidase IV activity and measures of body composition in apparently healthy people

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Abstract

Aim: Based on its regulatory action on glucagon-like peptide 1, dipeptidyl peptidase IV (DPP-IV) has increasingly been linked to Type 2 diabetes. However, there is no evidence as to how this normal modulatory enzyme leads to pathology. It is thought that DPP-IV is affected by the development of obesity, which is a common precursor to Type 2 diabetes. Little is known about the relationship between DPP-IV activity in plasma and specific body composition measures.

Main methods: In the current study, plasma DPP-IV activity and body composition measures were collected from 111 healthy subjects between the ages of 19 and 70 years old for analysis.

Key findings: The mean plasma DPP-IV activity was $35.9\text{U/L} \pm 12.3$, falling within normal reference value range presented by Durinx et al. DPP-IV activity was negatively correlated with absolute body fat mass, but absolute lean mass was positively correlated. Consistent with the findings, DPP-IV activity was also negatively correlated with absolute gynoid fat ($p = 0.0047$). DPP-IV activity did not have a significant correlation with absolute android fat mass, visceral adipose tissue, BMI, and age.

Significance: From these results, it can be concluded that high activity of DPP-IV is not indicative of pathology, and specific body composition components may influence soluble DPP-IV activity in the blood.

Keyword: Medicine

1. Introduction

Dipeptidyl-peptidase IV (DPP-IV), also known as CD26, is present in plasma as a soluble enzyme [1] and as a membrane-bound antigen on the surface of T-cell lymphocytes, on the endothelial layer of most blood vessels, and in the kidney [2]. DPP-IV plays an important role in immune function by activating T-cells [3], in controlling satiety by cleaving neuropeptide Y released by the hypothalamus [4], and in regulating insulin release via inactivating incretin hormones [5].

However, it is unclear how DPP-IV activity transitions from being a healthy modulator of a variety of important physiological mechanisms to pathological in people with diabetes. One hypothesis suggests DPP-IV activity is associated with the development of obesity. According to literature, it appears that DPP-IV activity has some connection to body composition in obese people [6, 7]. The evidence for this connection, however, is conflicting when looking at healthy individuals' DPP-IV activity and BMI as a measure of body composition [1, 8]. More specific body composition measures by the use of Dual X-Ray Absorptiometry (DEXA), which includes accurate measurements of fat mass and lean mass, could provide a better insight into the relationship between DPP-IV activity and body composition.

Previous literature suggests that obesity leads to increased rates of insulin resistance [9, 10]. However, not all fat masses are equal in terms of the relationship to insulin resistance. High visceral adipose tissue is known to increase the risk of obesity and diabetes [9]. In addition, high amounts of android fat is also related to higher risk of developing diabetes [11]. Currently, no studies address the relationship between DPP-IV activity and different fat compartments.

The purpose of this study was to identify the specific body composition factors with which the plasma DPP-IV activity was most highly correlated in apparently healthy subjects. It was hypothesized that DPP-IV activity is positively correlated with fat mass. We also expected a strong positive relationship between DPP-IV activity and visceral adipose tissue volume and android fat mass. We hypothesized that there would be no relationship between DPP-IV activity and gynoid fat, BMI, or lean mass.

2. Methods

2.1. Participant characteristics and ethics statement

For this study, 111 participants were recruited locally from the Auburn University area by the use of flyers around campus, the SONA system for the College of Education, and e-mails to classes in the School of Kinesiology (see Table 1 for a summary of participant characteristics). All participants were asked if they were diagnosed with diabetes and/or any cardiovascular or pulmonary diseases. They also completed a medical deferral list and the Full-length Donor History Questionnaire. Participants were included in the study if they were “apparently healthy,” which, for the purpose of this study, was defined as a self-reported absence of diagnosis of a clinical condition (i.e., participants answered “No” to all disease-based questions). Participants were excluded if they had any contraindications to participating in a blood draw, including diseases that would potentially cause the blood draw to be detrimental to either the participant or researcher. The study was submitted to and approved by the Institutional Review

Table 1. Participant characteristics.

	All (n = 111)	Men (n = 40)	Women (n = 71)
Age (yrs)	26 ± 10 (19–62)	27 ± 11 (19–60)	25 ± 9 (19–62)
Ethnicity (number of participants)	Asian = 3 Black = 14 Hispanic = 2 White = 92	Asian = 1 Black = 5 Hispanic = 0 White = 34	Asian = 2 Black = 9 Hispanic = 2 White = 58
Total mass (kg)	92.7 ± 21.8 (55.8–186.5)	98.0 ± 22.6 (62.0–186.5)	89.62 ± 20.8 (55.8–155.8)
BMI (kg/m ²)	23.8 ± 3.8 (17.5–37.1)	24.5 ± 3.7 (17.7–36.3)	23.4 ± 3.8 (17.5–37.1)
Absolute fat mass (kg)	20.1 ± 8.4 (4.5–53.2)	17.5 ± 8.9 (4.5–53.2)	21.6 ± 7.8 (9.0–49.6)
Visceral adipose tissue mass (kg)	0.3 ± 0.6 (0.0–3.9)	0.5 ± 0.8 (0.0–3.9)	0.2 ± 0.3 (0.0–1.4)
Visceral adipose tissue volume (cm ³)	340.4 ± 588.0 (0.0–4137.3)	557.7 ± 854.0 (13.4–4137.2)	217.9 ± 307.9 (0.0–1457.3)
Android fat mass (kg)	1.4 ± 0.9 (0.2–5.6)	1.4 ± 1.1 (0.2–5.6)	1.4 ± 0.8 (0.2–4.0)
Gynoid fat mass (kg)	3.6 ± 1.5 (0.6–8.2)	2.7 ± 1.4 (0.6–7.4)	4.0 ± 1.3 (1.7–8.2)
Absolute lean mass (kg)	48.4 ± 11.0 (30.1–85.9)	59.5 ± 8.9 (43.1–85.9)	42.1 ± 5.9 (30.1–59.3)
Dipeptidyl Peptidase Activity (U/L)	35.9 ± 12.3 (13.6–73.0)	43.3 ± 10.8 (21.0–73.0)	31.8 ± 11.1 (13.6–59.0)

Mean ± standard deviation (range).

Board at Auburn University prior to starting the study, and a written Informed Consent was obtained from all subjects.

2.2. Blood collection and analysis

Participants were asked to refrain from eating and drinking for at least an hour prior to any experimental measurements or blood sampling, as post-prandial DPP-IV activity does not change significantly after one hour [12]. Blood samples were collected using standard venipuncture in a prominent antecubital vein or a finger prick if the participants preferred not to have venipuncture. Plasma was separated by centrifugation for 10 minutes at 1,000 g and 4 °C. Plasma was subsequently stored in 0.5 mL aliquots at –80 °C until analysis.

2.3. Body composition analysis

On the same visit, the body composition of the participant was analyzed using a Lunar iDEXA machine (GE Healthcare, Fairfield, CT). This provides detailed information about relative and absolute fat in various compartments including visceral, android, and gynoid areas. The iDEXA also analyzed the participant for absolute and relative lean mass.

2.4. Plasma enzyme analysis

The plasma was analyzed for DPP-IV activity using a fluorometric assay developed by Scharpé et al. [13]. This assay measures the release of 4-methoxy-2-naphthylamine to determine the activity of DPP-IV and has a detection limit of 0.1U/L and a Km of 60.1 μM for the product glycyl-L-proline-4-methoxy-2-naphthylamine [14]. Enzymatic activity was defined as the amount of DPP-IV that cleaves 1 μmol of glycyl-L-proline-4-methoxy-2-naphthylamine per minute. DPP-IV activity was calculated using the equation below:

$$\text{Activity (U/L)} = [(S) \times V_A \times 1000 \times C_{st}] / [(T \times S_V \times (F))]$$

where S is the sample fluorescence minus the sample blank fluorescence, V_A is the total assay volume, 1000 is the correction factor for milliliters to liters, C_{st} is the standard concentration, T is the incubation time, S_V is the sample volume, and F is the standard fluorescence minus the standard blank fluorescence.

2.5. Statistical analysis

After determining the line of best fit, linear regression was used to determine the statistical significance of correlations between the enzymatic activity of DPP-IV in the plasma and the measures of age and BMI. A multiple linear regression model was used to determine the correlation between plasma DPP-IV activity and several specific body composition measures. The method of

backward elimination was applied to determine the significant factors of the model. All statistical analysis was performed using SAS 9.4 software (SAS Institute Inc., Cary, NC).

3. Results

3.1. Relationship to absolute lean and fat mass

After a multiple regression analysis of DPP-IV activity, lean mass, and fat mass, both measures of body composition were found to have a significant relationship with DPP-IV activity ($p < 0.0001$). A positive relationship was found between DPP-IV activity and absolute lean mass ($p < 0.0001$; Fig. 1A). Conversely, DPP-IV activity had a negative correlation with absolute fat mass ($p = 0.0147$; Fig. 1B). Together, the amount of absolute lean and fat mass had a moderate effect size with the activity of DPP-IV ($r = 0.4256$). When the interaction between absolute fat and lean mass was included, it was found to be insignificant and eliminated from the model.

3.2. Associations with different fat compartments in the body

Fat distribution was analyzed via multiple regression including the variables: absolute android fat (kg), absolute gynoid fat (kg), absolute visceral fat (kg), absolute lean mass (kg), and DPP-IV activity. Using backward elimination, the regression found only absolute lean mass and absolute gynoid fat to be significant ($p < 0.0001$). In this model, absolute lean mass was positively correlated to DPP-IV activity ($p = 0.0002$), but gynoid fat was negatively correlated with DPP-IV activity ($p = 0.0047$; Fig. 2A). When taken together, lean mass and gynoid fat mass had a moderate effect on DPP-IV activity ($r = 0.4433$), which was slightly higher than when using total fat mass. Both

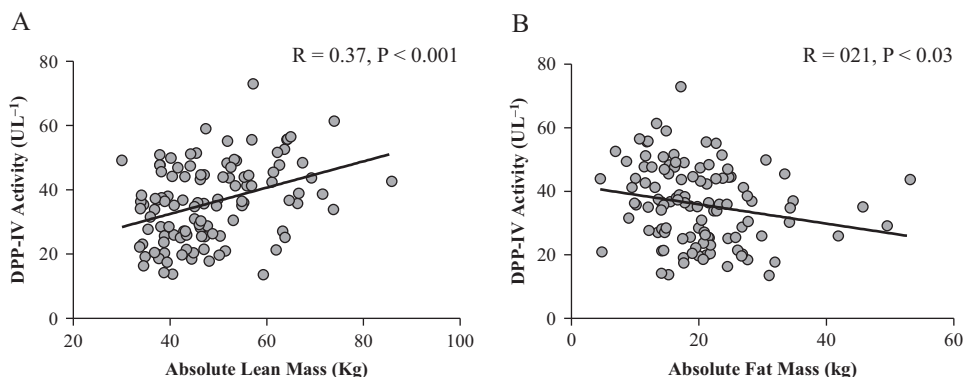


Fig. 1. The relationship among plasma DPP-IV activity and absolute and relative lean and fat mass. (A) DPP-IV activity vs. absolute lean mass. (B) DPP-IV activity vs. absolute fat mass.

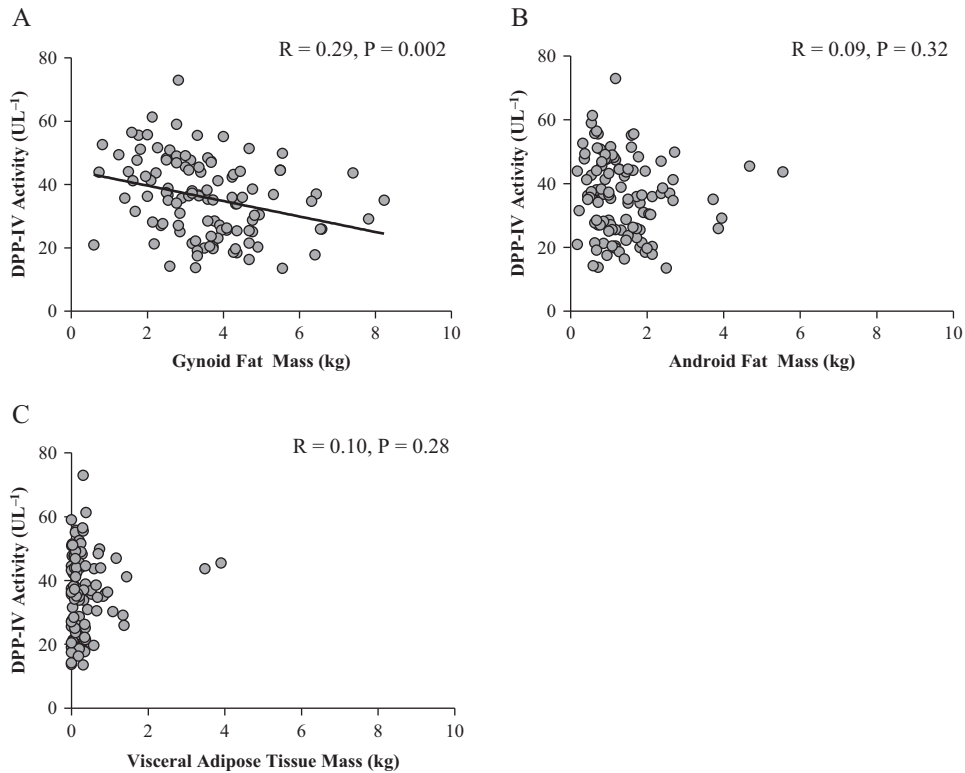


Fig. 2. The association between DPP-IV activity and different fat compartments. (A) DPP-IV activity vs. gynoid fat mass (B) DPP-IV activity vs. android fat mass. (C) DPP-IV activity and visceral adipose tissue mass.

absolute android fat (Fig. 2B) and absolute VAT (Fig. 2C) were eliminated from the model, due to having an insignificant relationship with DPP-IV activity.

3.3. Associations with age and BMI

The results from the linear regression analysis of age and DPP-IV activity showed that there was not a significant relationship between the two variables

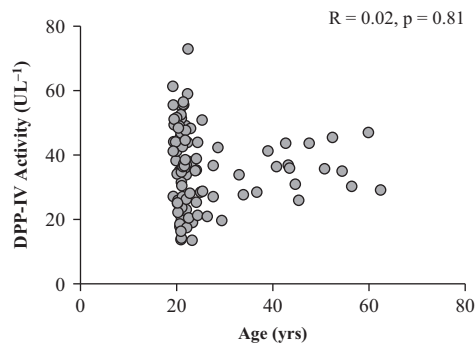


Fig. 3. The association between DPP-IV activity and age.

(Fig. 3). Additionally, no significant relationship was found between DPP-IV activity and BMI (Fig. 4). Fig. 5 shows the DPP-IV activity by weight classifications according to the participant's percent fat (A) and the same participants classified by their BMI (B). There was a significant relationship between BMI and percent fat in this group of participants ($p < 0.01$; Fig. 5C).

3.4. Post hoc analyses

Fig. 6 is DPP-IV versus absolute lean mass and shows that both men and women appear to fall on the same regression line.

4. Discussion

The original hypothesis of this study was that high enzymatic activity of DPP-IV would be associated with higher fat mass, and particularly with greater visceral adipose tissue. After analysis, several interesting findings were discovered. The main finding was that DPP-IV activity was negatively correlated with absolute fat mass, indicating that lower DPP-IV activity is associated with higher fat mass. We also found lean mass was positively correlated with the enzymatic activity of DPP-IV. DPP-IV activity was correlated with some specific fat compartments, while other measures such as BMI and visceral adipose tissue did not have an association with DPP-IV activity. This suggests that regulation of DPP-IV activity may be, in part, dependent on the individual's specific body composition.

4.1. Relationship to absolute lean and fat mass

Our data indicate that DPP-IV activity had a negative correlation with fat mass, suggesting people with more fat will have lower DPP-IV activity. However, fat cells do release DPP-IV and possibly contribute to the pool of plasma DPP-IV activity [15]. Lamers et al. [15] found that DPP-IV is released from adipose tissue explants, and adipocytes from obese patients release more DPP-IV when stimulated by inflammatory mediators. They also found that following

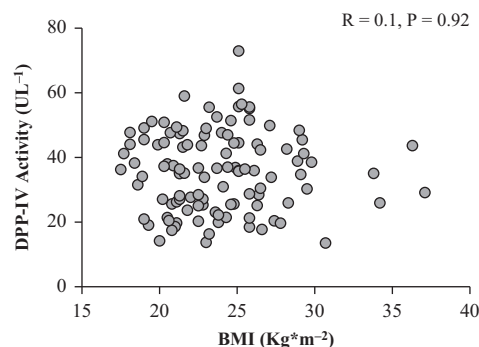


Fig. 4. The association between DPP-IV activity and BMI.

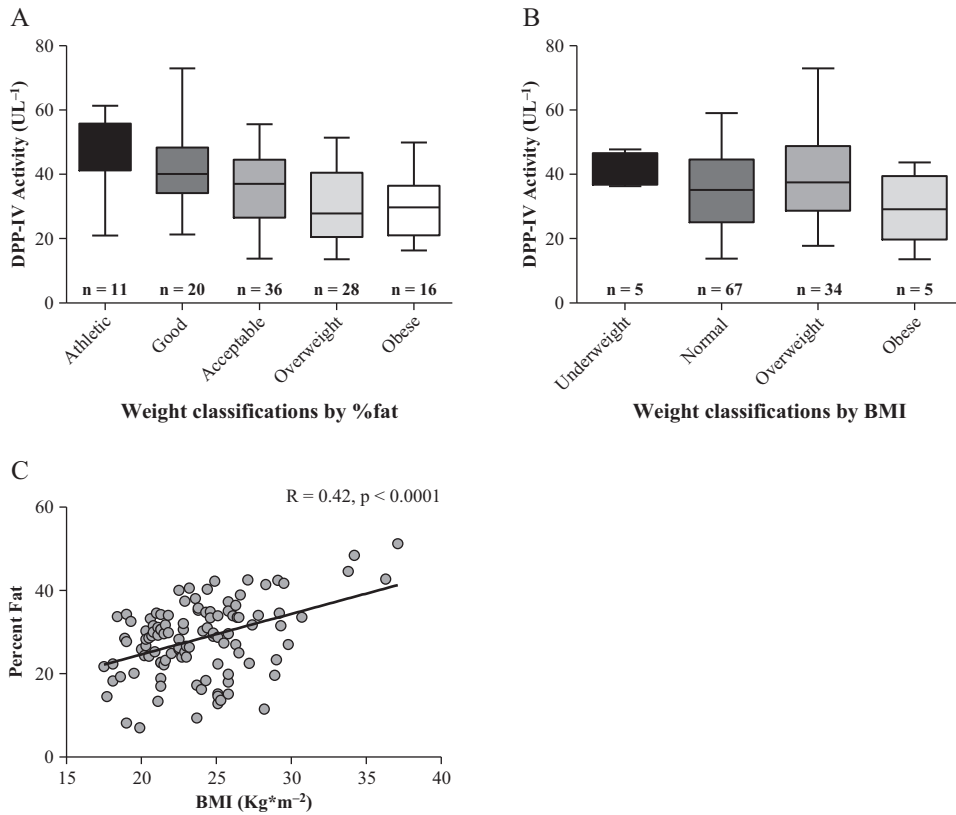


Fig. 5. Participants classified by Percent fat and BMI weight classifications. (A) DPP-IV activity by weight classifications according to the participant’s percent fat. (B) DPP-IV activity vs. BMI weight classifications. (C) Relationship between BMI and percent fat ($p < 0.01$).

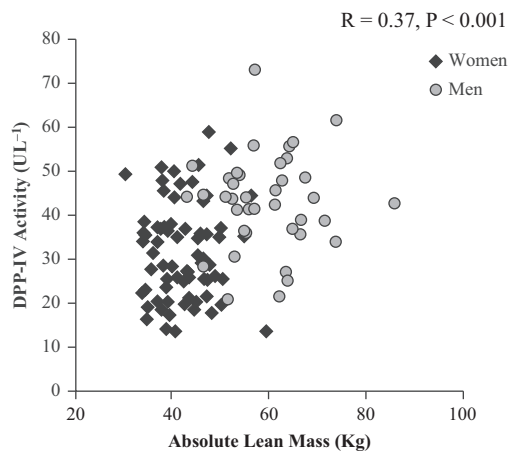


Fig. 6. The effect of gender on the relationships between DPP-IV activity and body composition. DPP-IV activity vs. absolute lean mass in males and females.

surgically-induced weight loss, this increase in DPP-IV release from adipocytes is normalized to the level of lean control subjects. However, these adipocytes were stimulated with growth factors that often are released with obesity, to achieve release of DPP-IV. It is possible that in non-obese individuals, fat cells experience little stimulation to release DPP-IV.

The most interesting finding from the current study was that DPP-IV activity was positively correlated with absolute lean mass. To our knowledge, this is the first time this was demonstrated and suggests that lean mass may play a role in modulating the soluble DPP-IV pool. A recent study showed DPP-IV is released from the skeletal muscle as a myokine in a contractile-related manner [16]. Also, studies on energy intake suggest lean mass is the most important factor for determining daily energy intake [17]. This may be significant for DPP-IV activity as DPP-IV acts to control the half-life of a number of signaling peptides related to food intake and satiety [5, 15, 18]. This along with the current data suggests that high DPP-IV activity may be due to muscle activity and may not indicate the development of pathology.

4.2. Associations with different fat compartments in the body

We hypothesized that DPP-IV activity would be positively correlated with visceral adipose tissue mass, based on previous research suggesting that visceral adipose tissue released more DPP-IV than subcutaneous adipose tissue [15, 19]. However, in the present study, VAT mass did not have a significant relationship with DPP-IV activity when accounting for the different compartments of fat. This could possibly be explained by the low amounts of visceral adipose tissue in the participants. Eleven individuals had visceral adipose tissue below detectable limits, and the mean value was 0.32 kg, which is much lower than values reported in other studies [20, 21]. It is possible that visceral adipose tissue under a certain threshold amount does not contribute significantly to the activity of whole body DPP-IV.

We also hypothesized that a positive correlation between DPP-IV activity and android fat would be present, while there would be no correlation with gynoid fat. However, DPP-IV activity was only significantly related to absolute gynoid fat when the model included distribution of centralized fat. This relationship was negative, indicating that individuals with less fat in the gynoid region are likely to have higher levels of plasma DPP-IV activity. In the present study, women had lower average DPP-IV activity compared to males, so it is possible that the relationship with gynoid fat is a sex effect rather than a fat mass effect. However, there is little information regarding sex and DPP-IV activity.

4.3. Associations with age and BMI

The results of the present study showed no significant correlation between DPP-IV activity and age. This is in contrast with previous literature showing lower DPP-IV activity in older adults, resulting in normal to elevated active GLP-1 levels [22]. A possible explanation is the above referenced participants were all classified as obese, therefore, the relationship with age in Meneilly et al. may be explained by their high fat mass, rather than age [22]. This is in agreement with the current data showing lower DPP-IV activity with increased fat mass. Although we had a wide range of ages in this study, most of our participants were in their 20's. This may also explain the lack of relationship between DPP-IV activity and age in the current study.

According to the current data, the lack of correlation between DPP-IV activity and BMI agrees with previous research, suggesting that BMI is not predictive of DPP-IV activity in the plasma [1, 12, 23]. While Kirino et al. showed relationships between DPP-IV activity and BMI [24], it should be noted that their study investigated Japanese students, while our study looked predominately at Caucasian American students. It is possible that ethnicity may play a role in the relationship between BMI and DPP-IV activity.

BMI can present an inaccurate representation of overall body composition. Athletic individuals with higher lean mass often have BMI's that indicate obesity, whereas body fatness using BMI is underestimated in individuals, such as older adults, who are prone to loss of lean mass [25, 26]. The results from this study were independent of the age of the participant, suggesting that accurate representation of body composition is valuable in understanding DPP-IV activity levels.

5. Conclusions

Dipeptidyl peptidase IV is an important regulatory enzyme that controls diverse functions such as insulin release, vasoconstriction, feeling satiated, and cell proliferation. However, we know little about how DPP-IV activity is regulated in the body. This study was an attempt to understand the relationships between specific body composition measurements and DPP-IV activity in apparently healthy individuals. We found that high absolute lean mass and low absolute fat mass have a moderate relationship with high DPP-IV activity. While this is a correlational study and does not present a cause and effect, it shows that more causative studies need to be performed to determine what controls DPP-IV activity in the blood, including muscular activity, sympathetic nerve activity, and stress [2, 16, 27, 28]. These findings also provide a basis for continued research in understanding what causes DPP-IV activity to change and why it becomes an important target for pharmaceutical management of diabetes.

Declarations

Author contribution statement

Leslie E. Neidert, Jeganathan Ramesh Babu, and Heidi A. Kluess: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Katherine S. Wainright, Chen Zheng: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Conflict of interest statement

The authors declare no conflict of interest.

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Additional information

Data associated with this study has been deposited at Dryad (<http://dx.doi.org/10.5061/dryad.2680t>).

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