Reduction in Endogenous Insulin Secretion Is a Risk Factor of Sarcopenia in Men with Type 2 Diabetes Mellitus

Ken-ichiro Tanaka, Ippei Kanazawa, Toshitsugu Sugimoto

Department of Internal Medicine 1, Shimane University Faculty of Medicine, Izumo 693-8501, Japan

Email addresses: K Tanaka: ken1nai@med.shimane-u.ac.jp; I Kanazawa: ippei.k@med.shimane-u.ac.jp; T Sugimoto: sugimoto@med.shimane-u.ac.jp

Corresponding author:

Ippei Kanazawa, MD, PhD

Department of Internal Medicine 1, Shimane University Faculty of Medicine, Izumo 693-8501, Japan

Phone: +81-853-20-2183 (ext. 2183), Fax: +81-853-23-8650

E-mail: ippei.k@med.shimane-u.ac.jp

Abstract

Sarcopenia has recently attracted widespread attention, because it increases risks of fall and bedridden. Although patients with type 2 diabetes mellitus (T2DM) are known to have lower muscle mass of limbs than healthy people, the mechanism is still unclear. We thus examined the association of muscle mass with parameters of endogenous insulin secretion such as fasting immunoreactive insulin (fIRI), fasting C-peptide immunoreactivity (fCPR), and daily urine CPR (U-CPR) in 191 men with T2DM. Muscle mass of arms and legs was evaluated by dual-energy x-ray absorptiometry, and we calculated relative skeletal muscle index (RSMI), which is useful for the diagnosis of sarcopenia. Multiple regression analyses adjusted for age, duration of T2DM, serum creatinine, HbA1c, and insulin like growth factor-I (IGF-I) showed that each parameter of endogenous insulin was significantly and positively correlated with muscle mass of arms and legs as well as RSMI ($p<0.05$). Moreover, logistic regression analyses adjusted for confounding factors mentioned above showed that each parameter of endogenous insulin was significantly lower in subjects with sarcopenia than those without it ($p<0.05$). In conclusion, reduction in endogenous insulin secretion is an independent risk factor of sarcopenia in men with T2DM.

Key words: Sarcopenia, Insulin Secretion, Diabetes mellitus, Skeletal muscle mass, IGF-I

Running head: Insulin and sarcopenia in men with T2DM

Introduction

With the aging of populations in the world, reduction in muscle mass has recently attracted widespread attention. Sarcopenia is a serious disease with the progressive loss of skeletal muscle mass and function, resulting in frailty and bedridden in elderly people. In recent days, sarcopenia has been recognized as one of diabetic complications [2]. Tajiri *et al* showed that the percentage of skeletal muscle of whole body and lower extremities was lower in 198 Japanese patients with type 2 diabetes mellitus (T2DM) than in 198 healthy subjects [22]. In addition, Park *et al* reported that elderly people with T2DM showed greater declines in muscle mass and strength of legs or extremities compared with those without T2DM [13, 14]. These findings indicate that patients with T2DM have excessive loss of appendicular lean mass compared with healthy subjects. However, the mechanism of sarcopenia seen in T2DM is still unclear.

Insulin regulates metabolism of carbohydrates by increasing the absorption of glucose from the blood to skeletal muscles and other tissues. Further, insulin has an anabolic effect on muscle through the uptake of amino acid into muscle tissues [3, 4]. The capacity of endogenous insulin secretion is known to eventually reduce even in T2DM; therefore, we hypothesized that endogenous insulin secretion might be associated with reduction in muscle mass in T2DM patients.

On the other hand, Insulin-like growth factor-I (IGF-I) is a hormone produced in the liver through the regulation by growth hormone or diet, and is associated with poor physical function or disability in elderly people. Previous studies have shown that IGF-I is associated with muscle growth

and hypertrophy [1, 10, 17], and that the low level of serum IGF-I is associated with low muscle strength and mobility in old women [5]. Insulin receptor substrate protein-1, one of substrates of the insulin and IGF-I receptors, is known to plays the main role in skeletal muscle [15]. Furthermore, endogenous insulin secretion is needed for hepatic expression and generation of IGF-I [7, 18]. Thus, to evaluate the association between endogenous insulin and muscle mass, we should consider the effect of IGF-I on the association.

In this study, we therefore investigated the independent association of serum IGF-I levels between the parameters of endogenous insulin and indices of muscle mass in elderly men with T2DM.

Material and Methods

Subjects

We consecutively screened 229 men with T2DM who admitted in Shimane University Hospital from 2007 to 2011. Thirty-eight men among them were excluded from this study due to insulin treatment, hepatic or renal dysfunction, and nutritional derangements that might cause change in indices of insulin in serum and urine as well as serum IGF-I. Finally, 191 Japanese men with T2DM (age; 60.2 ± 12.5 years) were included in this study. All subjects agreed to participate in this study and gave informed consent. This study was approved by the institutional review board of Shimane University Faculty of Medicine and complied with the Helsinki declaration.

Biochemical measurements

After overnight fasting, serum samples were collected, and urine sample was collected for 24 hours to measure daily urine CPR (U-CPR). Biochemical markers were measured by standard methods as previously described [8, 9]. Hemoglobin A1c (HbA1c) was determined by high performance liquid chromatography. The value for HbA1c was estimated as an NGSP (National Glycohemoglobin Standaridization Program) equivalent value calculated by the formula: HbA1c (%) = HbA1c (JDS) (Japan Diabetes Society) (%) + 0.4% [19]. Serum IGF-I was measured by RIA with [^{125}I]-IGF-I as a competitive radioligand and a polyclonal anti-human antibody [8]. Bound radioactivity was measured using a gamma counter and concentrations were determined relative to a standard curve prepared with recombinant

human IGF-I. The CV of IGF-I measurement was 2.28%.

Measurements of muscle mass by whole body dual-energy x-ray absorptiometry

Lean body mass of arms and legs as well as appendicular skeletal muscle mass (ASM) were evaluated by dual-energy x-ray absorptiometry (QDR-4500, Hologic co.). Relative skeletal muscle mass index (RSMI) was calculated by following formula; ASM/height^2, as previously described [6, 23]. The reference value of RSMI in Japanese men with sarcopenia (2 SD below the sex-specific means) was 6.87 kg/m^2 [16]. We regarded the stage of presarcopenia, which was characterized by low muscle mass, as sarcopenia [24], because the information about muscle strength and performance were not available in the present study.

Statistical analysis

Data are expressed as mean ± SD. Because HbA1c, fasting immunoreactive insulin (fIRI), fasting C-peptide immunoreactivity (fCPR), U-CPR, IGF-I, and muscle mass showed skewed distribution, they were transformed to logarithms before simple or multiple regression analysis. All analyses were carried out using statistical computer programs, StatView (Abacus Concepts, Berkeley, CA). A *p value* <0.05 was considered as significant.

Results

Background data of the subjects and simple correlations of muscular parameters with insulin parameters and serum IGF-I levels

Clinical characteristics of the subjects are shown in Table 1. The numbers of patient who had been taking sulfonylurea, metformin, alpha-glucosidase inhibitors, and pioglitazone were 75, 23, 30, and 16, respectively. First, we investigated simple correlations of muscular parameters with various parameters including serum insulin and IGF-I. As shown in Table 2, muscle mass of arms and legs and RSMI were significantly and negatively correlated with age and duration of diabetes. Furthermore, the muscular parameters were significantly and positively correlated with fIRI, fCPR, U-CPR, and IGF-I. In contrast, any muscle parameters were not correlated with HbA1c.

Multiple regression analysis between muscular and endogenous insulin parameters

Multiple regression analysis was then performed using indices of muscle mass as dependent variables and age, duration of T2DM, serum creatinine, log (HbA1c), log (IGF-I), and parameters of endogenous insulin secretion as independent variables (Table 3). Fasting IRI, fCPR, and U-CPR levels were significantly and positively associated with all muscular parameters.

Association of endogenous insulin parameters with the presence of sarcopenia

Next, we examined the association of demographic and biochemical parameters of subjects with and without sarcopenia. As shown in Table 4, age, duration of T2DM, and HbA1c were significantly higher in subjects with sarcopenia than those without it. In contrast, body mass index, IGF-I, and indices of endogenous insulin as well as muscle mass were significantly lower in subjects with sarcopenia than those without it. Further, logistic regression analyses adjusted for age, duration of T2DM, and serum creatinine showed that parameters of insulin such as fIRI, fCPR, U-CPR as well as serum IGF-I were significantly lower, and HbA1c was significantly higher in subjects with sarcopenia than those without sarcopenia (Table 5). In addition, the association between the presence of sarcopenia and parameters of endogenous insulin was still significant even after adjustment for IGF-I and HbA1c [for log(fIRI); odds ratio (OR)=0.14, 95% confidence interval (CI)=0.03-0.58, p=0.007; for log(fCPR), OR=0.04, 95%CI=0.01-0.36, p=0.004; for log(U-CPR), OR=0.22, 95%CI=0.06-0.76, p=0.016, respectively].

Discussion

Sarcopenia is the progressive loss of skeletal muscle mass and strength with a risk of adverse outcomes with aging. Although sarcopenia is known to be a diabetic complication, the mechanism of sarcopenia in T2DM is still unclear. Several studies have shown that high HbA1c and low IGF-I levels are associated with muscle weakness and the presence of sarcopenia [1, 5, 10, 17, 21]. The present study showed that endogenous insulin secretion and serum IGF-I levels were significantly and positively associated with muscular parameters and that low insulin parameters and IGF-I levels as well as high HbA1c levels were significantly associated with the presence of sarcopenia in men with T2DM. These findings are consistent with previous reports. Moreover, decreased endogenous insulin was associated with reduction in indices of muscle mass independently of HbA1c and IGF-I levels. The findings indicate that endogenous insulin plays an important role in the pathogenesis of sarcopenia in diabetic patients.

Maintaining β-cell function is very important for stable control of blood glucose levels. Furthermore, previous studies have shown that the capacity of endogenous insulin secretion is associated with progression of diabetic complications. Kuo *et al* reported a prospective study showing that residual insulin secretion provides important protection against the development of diabetic retinopathy in 585 T2DM patients [11]. In the present study, parameters of endogenous insulin were positively associated with muscular indices independently of age, duration of diabetes, serum creatinine, HbA1c and serum IGF-I levels. Further, parameters of endogenous insulin were independently and inversely associated

with the presence of sarcopenia in T2DM. In the present study, the prevalence rate of sarcopenia is 44.5%. Sanada *et al.* previously reported that the prevalence of class 2 sarcopenia is 56.7% in 70-85 aged Japanese men (16). The previous and our studies showed that the prevalence rate of sarcopenia seems to be higher in Japanese than Caucasian (16, 25, 26). Capacity of insulin secretion and degree of obesity in Asian are known to be different from Western people (27). The low level of endogenous insulin might cause the increased prevalence rates of sarcopenia in the present study. Taken together, these findings indicate that decreased endogenous insulin is an independent risk factor of sarcopenia and that maintaining endogenous insulin secretion may be very important to prevent sarcopenia.

In contrast, Shishikura *et al* showed that skeletal muscle mass index was negatively correlated with the log-transformed stimulated CPR values by glucagon loading testing in young T2DM patients under 65 years old [20]. Unlike our study, 77 subjects out of 138 were treated with insulin administration, and the mean HbA1c values of the subjects were around 10% in their study. Generally, glucagon loading test is considered to underestimate residual insulin secretion if glucotoxicity existed. Strengths of our study are that the number of the subjects is relatively larger than that of the previous study and that we excluded the patients received insulin therapy. In addition, we performed multiple regression analysis adjusted for serum IGF-I levels. On the contrary, more than 50% of our subjects have been treated with oral anti-diabetic medications. Thus, we cannot exclude the direct effects of hypoglycemic agents on muscle mass as well as serum IGF-I in T2DM. We therefore need to conduct further longitudinal studies to evaluate the causal relationship between the reduction in endogenous

insulin and the progression of muscle mass reduction in future.

Previous studies have shown that IGF-I has an anabolic effect on muscle tissues [1, 10, 17]. Moreover, McMahon *et al* showed that the combination of IGF-I and exercise was additive in maintaining the diameter of myofibers of the quadriceps muscles in mice [12]. The present study showed that serum IGF-I was positively associated with muscular indices and inversely with the presence of sarcopenia in men with T2DM. These findings confirm that serum IGF-I is important to increase muscle mass. Endogenous insulin secretion plays an important role in the secretion of IGF-I from the liver [7, 18]; therefore, endogenous insulin may directly affect muscle tissue and indirectly through increasing serum IGF-I levels.

In conclusion, this study for the first time showed that the levels of endogenous insulin were positively associated with indices of muscle mass independently of serum IGF-I in men with T2DM. These findings suggest that reduction in endogenous insulin is an independent risk factor for diabetes-related sarcopenia and that maintaining endogenous insulin is important to prevent it.

Conflict of interest

All authors (KT, IK and TS) have any conflict of interest.

Acknowledgements

Authors' roles: Conceived and designed the study: IK. Corrected and analyzed the data: KT and IK.

Contributed equipment/materials: TS. Wrote the paper: KT and IK. Approving final version: All authors.

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Table 1 Baseline characteristics of subjects

Number of patients	191
Age (years)	60.2 ± 12.5
Duration of diabetes (years)	9.8 ± 8.7
Body mass index (kg/m^2)	23.8 ± 3.9
Serum creatinine (mg/dL)	0.80 ± 0.22
HbA1c (NGSP) (%)	8.5 ± 2.2
IGF-I (ng/mL)	147.2 ± 50.0
fIRI (μU/mL)	5.9 ± 4.8
fCPR (μU/mL)	1.9 ± 0.8
U-CPR (μg/day)	77.0 ± 41.1
Muscle mass of arms (g)	5501.7 ± 1119.0
Muscle mass of legs (g)	15067.0 ± 3129.0
RSMI (kg/m^2)	7.45 ± 1.12

HbA1c, hemoglobin A1c; IGF-I, insulin-like growth factor-I; fIRI, fasting immunoreactive insulin; fCPR, fasting C-peptide immunoreactivity; RSMI, relative skeletal muscle mass index

Table 2 Correlations of muscular parameters with various variables

	log (Muscle mass of arms)		log (Muscle mass of legs)		log (RSMI)	
	r	p	r	p	r	p
Age	-0.51	<0.001	-0.59	<0.001	-0.46	<0.001
Duration of diabetes	-0.20	0.007	-0.20	0.007	-0.21	0.004
Body mass index	0.62	<0.001	0.65	<0.001	0.73	<0.001
Serum creatinine	-0.11	0.149	-0.06	0.390	-0.05	0.526
log (HbA1c)	0.36	0.958	-0.07	0.361	-0.12	0.103
log (IGF-I)	0.00	<0.001	0.39	<0.001	0.37	<0.001
log (fTRI)	0.34	<0.001	0.35	<0.001	0.36	<0.001
log (fCPR)	0.38	<0.001	0.41	<0.001	0.43	<0.001
log (U-CPR)	0.27	<0.001	0.27	<0.001	0.30	<0.001

RSMI, relative skeletal muscle mass index; fIRI, fasting immunoreactive insulin; fCPR, fasting C-peptide immunoreactivity; U-CPR, urinary CPR; IGF-I, insulin-like growth factor-I

Table 3 Multiple regression analyses between parameters of muscular and endogenous insulin

	log (Muscle mass of arms)		log (Muscle mass of legs)		log (RSMI)	
	β	p	β	p	β	p
log (fIRI)	0.27	<0.001	0.26	<0.001	0.30	0.001
log (fCPR)	0.30	<0.001	0.32	<0.001	0.35	<0.001
log (U-CPR)	0.23	<0.001	0.24	<0.001	0.28	<0.001

RSMI, relative skeletal muscle mass index; fIRI, fasting immunoreactive insulin; fCPR, fasting C-peptide immunoreactivity; U-CPR, urinary CPR
Adjusted for age, duration of T2DM, serum creatinine, log (HbA1c), and log (IGF-I)

Table 4 Demographic and biochemical parameters of subjects with and without sarcopenia (RSMI<6.87 kg/m^2)

	with sarcopenia	without sarcopenia	*P*
Number of patients	85	106	
Age (years)	65.0 ± 9.6	56.3 ± 13.2	<0.001
Duration of diabetes (years)	11.5 ± 9.3	8.4 ± 8.0	0.018
Body mass index (kg/m^2)	21.4 ± 2.5	25.7 ± 3.8	<0.001
Serum creatinine (mg/dL)	0.78 ± 0.21	0.81 ± 0.22	0.490
HbA1c (NGSP) (%)	8.9 ± 2.2	8.1 ± 2.2	0.024
IGF-I (ng/mL)	129.6 ± 44.8	161.0 ± 49.8	<0.001
fIRI (μU/mL)	5.0 ± 5.5	6.6 ± 4.2	0.030
fCPR (μU/mL)	1.6 ± 0.7	2.1 ± 0.9	<0.001
U-CPR (μg/day)	63.0 ± 40.0	88.5 ± 38.4	<0.001
Muscle mass of arms (g)	4683.5 ± 712.8	6157.8 ± 939.6	<0.001
Muscle mass of legs (g)	12755.8 ± 1723.7	16920.2 ± 2747.5	<0.001
RSMI (kg/m^2)	6.53 ± 0.60	8.18 ± 0.87	<0.001

Data are means ± SD. *P* values were calculated using Student's t-test or χ^2 tests.

Table 5 The association of fIRI, fCPR, U-CPR, IGF-I, and HbA1c with the presence of sarcopenia in logistic analyses

	OR (95% CI)	*p*
log (fIRI)	0.13 (0.032-0.546)	0.005
log (fCPR)	0.04 (0.005-0.327)	0.002
log (U-CPR)	0.17 (0.051-0.580)	0.005
log (IGF-I)	0.08 (0.007-0.887)	0.040
log(HbA1c)	13.08 (0.552-309.806)	0.046

fIRI, fasting immunoreactive insulin; fCPR, fasting C-peptide immunoreactivity; U-CPR, urinary CPR; IGF-I, insulin-like growth factor-I

Adjusted for age, duration of T2DM, serum creatinine

OR, odds ratio; CI, confidence interval