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Obesity parameters, physical activity, and physical fitness are correlated with serum dipeptidyl peptidase IV activity in a healthy population

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Abstract

Objective: To determine whether obesity, physical fitness, and physical activity parameters are associated with the enzymatic activity of serum dipeptidyl peptidase IV (sDPPIV) in a sample of healthy women and men.

Design and methods: We have correlated parameters of obesity, physical fitness, and physical activity with sDPPIV activity in 374 healthy subjects (age: 60.7 ± 6.9 years, body mass index: 26.1 ± 4.1 kg/m^2). Enzymatic activity was analyzed using spectrofluorimetry, body composition was assessed by impedanciometry, physical fitness data were obtained using the Senior Fitness Test, and physical

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activity data were collected by accelerometer. Pearson's partial correlation analysis was applied to determine the relationship between DPPIV activity and the rest of parameters and significantly correlated variables were introduced into linear regression models to predict DPPIV.

Results: Serum DPPIV activity was negatively associated with obesity parameters such as body mass ($r = -0.112$), body mass index (BMI) ($r = -0.147$), waist circumference ($r = -0.164$), waist-to-hip ratio (-0.104), and percentage of fat mass ($r = -0.185$). Serum DPPIV activity was positively associated with cardiovascular fitness ($r = 0.138$), total amount of physical activity ($r = 0.153$), and time spent doing light exercise ($r = 0.184$). Regression models revealed sex differences in enzyme activity with overall activity higher in women than in men ($\beta = 0.437$, $p < 0.001$). Further, percent fat mass was an independent negative predictor of DPPIV activity ($\beta = -0.184$, $p = 0.001$). Serum DPPIV activity was positively predicted based on the amount of time spent doing light physical activity ($\beta = 0.167$, $p = 0.001$).

Conclusion: Our results demonstrate that sDPPIV activity is positively associated with healthier parameters regarding fatness, fitness and physical activity.

Keywords: Metabolism, Physiology, Endocrinology

1. Introduction

Dipeptidyl-peptidase IV (DPPIV) is a type II membrane-bound glycoprotein that can be released into body fluids [1]. The major source of and mechanism(s) regulating circulating DPPIV remain unknown [2, 3]. Almost 50 peptides (growth factors, hormones, and neuropeptides) have been identified as substrates for DPPIV. Its enzymatic action can generate biologically active peptides from precursors, convert active peptides to different receptor specificity, or inactivate peptides [4]. This enzyme plays known roles in immune function [5], controlling satiety in the hypothalamus [6], regulating insulin release, and controlling glucose homeostasis [7]. In addition, serum DPPIV (sDPPIV) has endocrine effects [8] on inflammation and cardiovascular regulation [9].

Indeed, DPPIV is expressed in adipocytes and may impair insulin sensitivity directly in fat tissue and in skeletal and smooth muscle cells [2]. Serum DPPIV levels are negatively associated with the accumulation of visceral fat and the presence of metabolic syndrome in men with type 2 diabetes [10]. However, it is unclear how DPPIV activity transitions from being a healthy modulator of a variety of important physiological mechanisms to an enzyme associated with pathological conditions. Some studies suggest that high DPPIV levels are associated with the development of obesity, while others show that lower DPPIV activity is related to increased fat mass [3]. Yet, the majority of studies conducted thus far have involved small sample sizes of obese subjects [1, 2, 10, 11, 12, 13].

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Over the last decade, mounting evidence has underscored the importance of crosstalk between skeletal muscle, adipose tissues, and organs such as liver; in particular, this crosstalk involves communication via myokines regarding the energy demands of active muscle [14]. A myokine function has been proposed for DPPIV in which its endocrine role could be related to exercise response; indeed, some authors have hypothesized that DPPIV decreases following exercise [11]. Physical activity, thought to positively influence energy balance by increasing energy expenditure, has positive effects on skeletal muscle metabolism and insulin sensitivity [15]. Exercise training is also well known to improve physical fitness and to combat chronic diseases and aging associated disorders [16], however, it is controversial whether physical fitness or physical activity are the best predictors of individual health [17]. Further, the relative and joint contributions of fatness, fitness, and physical activity to health remain poorly understood [18].

Sex differences play a role in the link between obesity and metabolic syndrome and cardiovascular diseases [19]. The associations between hypertension [20] and cardiovascular risk [21] with serum peptidase activity, including DPPIV [22], have been found to differ depending on sex [20, 21, 22] and age [20, 21]. Therefore, the influence of sex in the relationship between DPPIV and cardiovascular risk factors cannot be ruled out.

Given the interaction between physical activity/fitness and obesity, and the role of DPPIV as a myokine, we sought to determine whether obesity, physical fitness, and physical activity parameters are associated with the enzymatic activity of sDPPIV in a sample of healthy women and men.

2. Methodology

2.1. Participants

Participants in this study comprised 374 healthy volunteer individuals recruited to participate in a Project about Alzheimer's Disease in Gipuzkoa, Spain (GAP). Participants had no history of cardiovascular, proliferative, or central nervous system degenerative disease. The average age was 60 $\pm$ 6.9 years (209 females, 60.1 $\pm$ 7.0 years; 165 males, 60.1 $\pm$ 7.0 years). All participants underwent thorough medical examination and were excluded if they presented chronic pathologies involving drug treatment, such as estrogen replacement therapy, hormonal contraceptives or testosterone, or any other significant neurologic, systemic, or psychiatric disorder that could cause cognitive impairment, as well as women who were pregnant or breastfeeding.

All participants were informed of the extent of the study and signed an informed consent document approved by the Ethical and Scientific Committees (CEIC Euskadi PGA-2).

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2.2. Serum sample preparation

Blood samples (10 mL) were collected by venipuncture into heparinized tubes after fasting overnight. Following centrifugation (1500 rpm, 10 min), 0.5 mL serum samples were separated and stored frozen at −80 °C until DPPIV enzyme activity analysis.

2.3. Dipeptidyl peptidase IV enzyme activity

Dipeptidyl peptidase IV (EC 3.4.14.5) activity in serum samples was measured by spectrofluorimetry in a microplate reader fluorometer FLUOstar OPTIMA (BMG Labtech). All samples were analyzed in triplicate and incubated at 37 °C in microplates for 30 min. Each well contained 10 μL of serum and 190 μL of assay buffer (Tris-HCl 50 mM, 2 mM H-Gly-Pro-β-naftilamide (Bachem) pH 8.3). Blanks and controls were also included in each plate.

The incubation time was based on preliminary assays to assess the linearity of the reaction over time as well as protein content. When introduced into the fluorimeter, specific excitation (340 nm) and emission (410 nm) filters were selected. The enzyme activity was expressed as units of enzyme activity per milliliter of serum (U/mL). One unit of activity is the amount of enzyme required to release 1 pmol of fluorescent product per minute. Specific inhibition assays in the presence of Diprotin A 2 mM (Bachem) were performed to ensure appropriate measurement of DPPIV activity.

2.4. Anthropometric measurement

Body mass (kg), height (cm) were measured using height boards, weighing scales (Añó Sayol SL, Barcelona, Spain). Waist and hip circumference (cm) were measured with a non-elastic anthropometric tape measure to the nearest 0.1 cm. The body mass and height of the participants were then used to calculate body mass index (BMI) in kg/m^2. All measurements were taken by the same person following the standards of the International Society for the Advancement of Kinanthropometry [23]. Body composition (percent of muscle mass, percent of fat mass, and percent of fat-free mass) were determined using a bioimpedance meter (InBody 230, InBody Co. Ltd.).

2.5. Measurement of physical fitness data

Physical fitness was measured using the Senior Fitness Test (SFT) battery, which is designed to assess physical parameters associated with different components of physical fitness [24]. For lower body and upper body flexibility assessment, *Chair Sit-&-Reach* and *Back Scratch* tests were performed. Muscle strength was determined using *Chair Stand* and *Arm Curl* tests for lower and upper limbs, respectively.

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To determine power, speed, agility, and dynamic balance *2.45 m Timed Up-&-Go* test was used and cardiorespiratory fitness was assessed with the *6-min Walk* test (6MWT).

2.6. Measurement of physical activity

To accurately measure physical activity data, we used accelerometers that record active and sedentary periods during everyday life. Accelerometers offer a number of desirable features for monitoring human movement: amount, duration, frequency, and intensity of movement [25]. Accelerometry was performed using the Actigraph GT3X model (Actigraph LLC). The monitor was worn on the hip with a belt for a seven-day period; participants were asked to remove it only for sleeping and bathing. Data files recorded on the accelerometers were downloaded and processed using Actilife software (version 5, Actigraph, 2011). Only days in which the monitors were worn for ten or more hours were considered valid, and at least three days of data were required to validate the recorded data [26]. Non-wear time was defined by an interval of at least 60 consecutive minutes of zero activity intensity counts, with tolerance for 2 min of counts between 0 and 50. The summary variables presented in this analysis are as follows: number of steps per day, mean counts per minute (CPM), and number of minutes per day spent in intensity-specific categories. Sedentary time was defined as all activity below 100 CPM. The classification developed by Freedson [27] was followed for the rest of the cut-off points to classify physical activity as light (100−1951 CPM), moderate (1952−5724 CPM), or vigorous ($\geq$5725 CPM). Moderate to Vigorous Physical Activity (MVPA) was viewed as the sum of minutes spent in moderate and vigorous physical activity ($\geq$1952 CPM).

2.7. Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc., version 24, IBM). A normality test was performed (Kolmogorov-Smirnov) for all the variables analyzed, for all participants, and for female and male groups. Depending on the normality of the dependent variable, the differences between groups were compared using the Student's t-test or Mann−Whitney U test, respectively, to analyze sex differences. The data are presented as mean $\pm$ standard deviation (SD).

Pearson's partial correlation analysis, with age and diabetes medication status (and sex when the whole group was analyzed) as the control variables, was carried out to determine the relationship between DPPIV activity and the rest of the parameters. When raw results did not show a normal distribution, the normality of their log or square root was analyzed. If data were not normally distributed, they were ranked to perform partial correlations. The results are presented as Pearson's correlation coefficient (r). The differences were considered to be statistically significant when $p < 0.05$.

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Variables that were significantly correlated ($p < 0.05$) by Pearson correlation with DPPIV serum activity were introduced into linear regression models to find the independent positive or negative association of the variable with enzymatic activity. Backward selection was used to select the models that best explained the variability of the dependent variables.

3. Results

3.1. Sex differences

Mean sDPPIV activity, age, anthropometric characteristics, physical fitness, and physical activity data for all participants, as well as separately for men and women, are described in Table 1. A Student's t-test or Mann-Whitney U test were applied to analyze sex-related differences (significance $p < 0.05$).

Serum DPPIV activity was significantly higher in women than in men ($p < 0.001$). Body mass, height, BMI, waist circumference, percent of muscle mass, and percent of fat-free mass were higher in men than in women. However, percent of fat mass was higher for women than men and there were no significant sex-dependent differences in age or waist-to-hip ratio.

Upper and lower limb flexibility was higher for women ($p < 0.001$ both). However, men showed better arm strength ($p = 0.014$) and walked farther in the six-minute walk test ($p < 0.001$).

The most significant difference between sexes was found in the amount of time spent in vigorous activity; men scored significantly higher in this area than women ($p = 0.007$). However, women spent significantly more time doing light activities during the day ($p = 0.041$).

3.2. Correlations

Serum DPPIV activity was assessed for correlation with anthropometric parameters (Table 2). Pearson's coefficient showed a negative correlation between enzyme activity and body mass ($r = -0.112$, $p = 0.032$), BMI ($r = -0.147$, $p = 0.005$), waist circumference ($r = -0.164$, $p = 0.002$), waist-to-hip ratio ($r = -0.104$, $p = 0.046$), and percent of fat mass ($r = -0.185$, $p < 0.001$) in all participants. The same significant correlations were found in men (body mass, $r = -0.188$, $p = 0.017$; BMI, $r = -0.195$, $p = 0.013$; waist circumference, $r = -0.206$, $p = 0.009$; waist-to-hip ratio, $r = -0.172$, $p = 0.030$; percent of fat mass, $r = -0.211$, $p = 0.007$). In women, the only correlation with sDPPIV was found when the percent of fat mass was analyzed, which was negatively correlated with enzyme activity ($r = -0.160$, $p = 0.023$).

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Table 1. Anthropometric characteristics, physical fitness, and physical activity among healthy individuals (mean ± SD).

Variables	All subjects		Women		Men		*p*
	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	
sDPPIV (UP/mL)	33739 ± 7180	*369*	36085 ± 6950	*206*	30773 ± 6335	*163*	<**0.001**^{t}
Anthropometric characteristics							
Age (years)	60.7 ± 6.9	*373*	60.1 ± 7.0	*209*	61.4 ± 6.8	*164*	0.068^{U}
Body mass (kg)	71.7 ± 14.0	*374*	64.3 ± 10.6	*209*	81.3 ± 11.8	*165*	<**0.001**^{t}
Height (cm)	165.5 ± 8.4	*374*	160.4 ± 6.0	*209*	172.0 ± 6.3	*165*	<**0.001**^{U}
BMI (kg/m^2)	26.1 ± 4.1	*374*	25.0 ± 4.1	*209*	27.5 ± 3.7	*165*	<**0.001**^{t}
Waist circumference (cm)	92.5 ± 11.2	*373*	88.3 ± 10.3	*208*	97.9 ± 9.9	*165*	<**0.001**^{t}
Waist-to-hip ratio	0.95 ± 0.05	*373*	0.95 ± 0.05	*209*	0.94 ± 0.04	*165*	0.121^{U}
Muscular mass (%)	27.3 ± 6.1	*374*	22.8 ± 2.8	*209*	33.2 ± 3.9	*165*	<**0.001**^{U}
Fat mass (%)	30.6 ± 8.1	*374*	33.5 ± 7,9	*209*	26,8 ± 6,7	*165*	<**0.001**^{t}
Fat-free mass (%)	49.5 ± 10.1	*374*	42.1 ± 4.7	*209*	58.9 ± 6.6	*165*	<**0.001**^{U}
Physical fitness data							
Chair Sit-&-Reach (cm +/−)	0.36 ± 10.8	*367*	3.31 ± 9.5	*205*	−3.38 ± 11.3	*162*	<**0.001**^{U}
Back Scratch (cm +/−)	−1.02 ± 8.9	*360*	1.80 ± 6.7	*201*	−4.59 ± 10.1	*159*	<**0.001**^{U}
Chair stand (stands)	16.2 ± 4.5	*368*	15.9 ± 4.2	*205*	16.55 ± 4.9	*163*	0.248^{U}
Arm Curl (reps)	16.7 ± 4.6	*368*	16.1 ± 4.3	*205*	17.38 ± 4.8	*163*	**0.014**^{U}
8-Ft Up-&-Go (s)	7.02 ± 1.5	*368*	6.94 ± 1.3	*204*	7.13 ± 1.8	*164*	0.215^{U}
6-Min Walk (m)	578.4 ± 86.0	*366*	560.5 ± 76.1	*204*	600.9 ± 92.5	*162*	<**0.001**^{U}
Physical activity data							
CPM	741.5 ± 221.6	*332*	731.3 ± 195.9	*188*	754.8 ± 251.4	*144*	0.740^{U}
Steps/day	10315 ± 3594	*332*	10150 ± 3349	*188*	10530 ± 3892	*144*	0.632^{U}
Sedentary (min/day)	503.3 ± 82.8	*332*	501.12 ± 75.9	*188*	506.3 ± 91.3	*144*	0.425^{U}
Light (min/day)	275.8 ± 70.3	*332*	281.7 ± 71.6	*188*	268.1 ± 68.1	*144*	**0.041**^{U}
Moderate (min/day)	55.1 ± 28.8	*332*	52.6 ± 27.1	*188*	58.3 ± 30.7	*144*	0.191^{U}
Vigorous (min/day)	2.35 ± 8.2	*332*	1.19 ± 3.1	*188*	4.00 ± 12.6	*144*	**0.007**^{U}
MVPA (min/day)	57.6 ± 31.2	*332*	53.8 ± 27.9	*188*	62.5 ± 34.5	*144*	0.064^{U}

sDPPIV = Serum dipeptidyl peptidase IV; BMI = body mass index; CPM = counts per min.; MVPA = moderate to vigorous physical activity, $^{(t)}$Student's *t*-test, $^{(U)}$Mann-Whitney test.
Bold values indicate $p<0.05$.

Table 3 shows correlations between sDPPIV activity and physical fitness variables of the participants. In all participants ($r = 0.138$, $p = 0.009$), as well as when women ($r = 0.144$, $p = 0.041$) and men ($r = 0.181$, $p = 0.023$) were analyzed separately, significant positive correlations were found between enzyme activity and 6MWT scores.

Positive associations between physical activity data (Table 4) and sDPPIV activity were found with CPM ($r = 0.153$, $p = 0.006$) and the time spent in light intensity physical activity ($r = 0.184$, $p = 0.001$), in all participants. In women, the correlation

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Table 2. Pearson's correlation coefficients (r) between anthropometric characteristics and sDPPIV enzymatic activity.

Variable	Partial correlation					
	All subjects[a]		Women[b]		Men[c]	
	r	*p*	*r*	*p*	*r*	*p*
Body mass (kg)	**−0.112**	**0.032**	−0.050	0.480	**−0.188**	**0.017**
Height (cm)	0.056	0.284	0.103	0.149	−0.046	0.574
BMI (kg/m^2)	**−0.147**	**0.005**	−0.096	0.170	**−0.195**	**0.013**
Waist circumference (cm)	**−0.164**	**0.002**	−0.114	0.106	**−0.206**	**0.009**
Waist-to-hip ratio	**−0.104**	**0.046**	−0.074	0.292	**−0.172**	**0.030**
Muscular mass (%)	0.048	0.359	0.135	0.053	−0.049	0.536
Fat mass (%)	**−0.185**	**<0.001**	**−0.160**	**0.023**	**−0.211**	**0.007**
Fat-free mass (%)	0.041	0.436	0.128	0.069	−0.056	0.478

[a] Controlled by sex, age, and medication for diabetes. N = 374.
[b, c] Controlled by age and medication for diabetes. [b]N = 206, [c]N = 163.
sDPPIV = Serum dipeptidyl peptidase IV; BMI = body mass index.
Bold values indicate p<0.05.

Table 3. Pearson's correlation coefficients (r) between physical fitness and sDPPIV enzymatic activity.

Variable	Partial correlation					
	All subjects[a]		Women[b]		Men[c]	
	r	*p*	*r*	*p*	*r*	*p*
Chair Sit-&-Reach (cm +/−)	0.083	0.115	0.024	0.739	0.106	0.186
Back Scratch (cm +/−)	0.080	0.131	0.096	0.180	0.001	0.989
Chair stand (no. of stands)	0.009	0.871	−0.068	0.336	0.132	0.098
Arm Curl (no. of reps)	0.073	0.164	0.020	0.777	0.130	0.103
8-Ft Up-&-Go (s)	−0.016	0.764	−0.058	0.415	0,005	0.949
6-Min Walk (m)	**0.138**	**0.009**	**0.144**	**0.041**	**0.181**	**0.023**

[a] Controlled by sex, age, and medication for diabetes. N = 374.
[b, c] Controlled by age and medication for diabetes. [b]N = 206, [c]N = 163.
sDPPIV = Serum dipeptidyl peptidase IV.
Bold values indicate p<0.05.

between enzyme activity and CPM (r = 0.161, p = 0.029) and light intensity activity (r = 0.207, p = 0.005) reached statistical significance, while in men, CPM was positively correlated with enzyme activity (r = 0.167, p = 0.049) and sedentary activity was negatively correlated with DPPIV activity (r = −0.178, p = 0.035).

3.3. Regression models

Linear regression models were built to ascertain the influence of the studied parameters on sDPPIV activity. Table 5 shows the regression model for the whole sample.

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Table 4. Pearson's correlation coefficients (r) between physical activity and sDPPIV enzymatic activity.

Variable	Partial correlation					
	All subjects[(a)]		Women[(b)]		Men[(c)]	
	r	*p*	*r*	*p*	*r*	p
CPM	**0.153**	**0.006**	**0.161**	**0.029**	**0.167**	**0.049**
Steps/day	0.104	0.062	0.116	0.117	0.117	0.167
Sedentary (min/day)	−0.101	0.170	−0.037	0.618	**−0.178**	**0.035**
Light (min/day)	**0.184**	**0.001**	**0.207**	**0.005**	0.155	0.067
Moderate (min/day)	0.059	0.285	0.112	0.129	0.023	0.785
Vigorous (min/day)	0.023	0.685	0.042	0.572	0.054	0.530
MVPA (min/day)	0.088	0.115	0.118	0.111	0.059	0.491

[(a)] Controlled by sex, age, and medication for diabetes. N = 374.
[(b, c)] Controlled by age and medication for diabetes. [(b)]N = 206, [(c)]N = 163.
sDPPIV = Serum dipeptidyl peptidase IV; BMI = body mass index.
CPM = counts per min; MVPA = moderate to vigorous physical activity.
Bold values indicate p<0.05.

Table 5. Linear regression model built with sDPPIV activity as the dependent variable and sex, age, medication for diabetes, percent of fat mass, 6-min walk, CPM, and light activity as independent variables.

	B	SE	95% confidence limits		Standard coefficients (β)	t	*p* value
			Lower	Upper			
(Constant)	12662	4500	3808	21516		2.814	**0.005**
Sex	6312	805.9	4726	7897	0.437	7.832	**<0.001**
Age	113.9	53.29	9.14	218.8	0.110	2.139	**0.033**
% fat mass	−163.0	49.7	−260.9	−65.16	−0.184	−3.277	**0.001**
Light activity	564.1	169.7	230.1	898.1	0.167	3.323	**0.001**

ANOVA R^2: 0.186; $p < 0.001$.
B: Non-standarized coefficient; SE: standard error; t: *t*-test statistics; p value indicates the significance of an ANOVA checking the value of the predictive model; R^2: coefficient of determination.
sDPPIV = Serum dipeptidyl peptidase IV; CPM = counts per min.
Bold values indicate p<0.05.

The starting model included sDPPIV activity as the dependent variable and sex, age, medication for diabetes, percent of fat mass, 6MWT, CPM, and time spent in light activity as independent variables. In the model that showed the highest final coefficient of determination ($R^2 = 0.186$, $p < 0.001$), sex, age, and minutes of light activity were positive predictors of sDPPIV activity. Interestingly, percent of fat mass was a negative predictor of enzyme activity.

In the model built with women's results, age, medication for diabetes, percent of fat mass, 6MWT results, CPM, and min of light activities were included as independent

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Table 6. Linear regression model built with sDPPIV activity in women as the dependent variable. The model includes age, medication for diabetes, percent of fat mass, 6-min walk, CPM, and light activity as independent variables.

	B	SE	95% confidence limits		Standard coefficients (β)	t	*p* value
			Lower	Upper			
(Constant)	19090	4862	9495	28684		3.926	<**0.001**
Age	267.1	69.9	129.2	405.1	0.270	3.821	<**0.001**
% fat mass	−143.2	62.2	−266.1	−20.3	−0.162	−2.300	**0.023**
Light activity	20.2	6.82	6.78	33.7	0.207	2.967	**0.003**

ANOVA R^2: 0.106; $p < 0.001$.
B: Non-standardized coefficient; SE: standard error; t: *t*-test statistics; p value indicates the significance of an ANOVA checking the value of the predictive model; R^2: coefficient of determination.
sDPPIV = Serum dipeptidyl peptidase IV; CPM = counts per min.
Bold values indicate $p<0.05$.

Table 7. Linear regression model built with sDPPIV activity in men as the dependent variable. The model includes age, medication for diabetes, percent of fat mass, 6-min walk, CPM, and sedentary activity as independent variables.

	B	SE	95% confidence limits		Standard coefficients (β)	t	*p* value
			Lower	Upper			
(Constant)	39012	2266	34530	43494		17.210	<**0.001**
% fat mass	−234.5	75.4	−383.7	−85.2	−0.251	−3.107	**0.002**
Sedentary activity	−11.3	5.12	−21.4	−1.16	−0.178	−2.204	**0.029**

ANOVA R^2: 0.082; $p = 0.001$.
B: Non-standardized coefficient; SE: standard error; t: *t*-test statistics; p value indicates the significance of an ANOVA checking the value of the predictive model; R^2: coefficient of determination.
sDPPIV = Serum dipeptidyl peptidase IV; CPM = counts per min.
Bold values indicate $p<0.05$.

variables (Table 6). Age and min of light activity resulted as positive predictors for sDPPIV activity, while the coefficient for the percent of fat mass was negative ($R^2 = 0.106$, $p < 0.001$).

Finally, in men, age, medication for diabetes, percent of fat mass, 6MWT results, CPM, and min of sedentary activity were included as independent variables (Table 7). In the last model, percent of fat mass and time spent in sedentary activities were found to be negative predictors of enzymatic activity ($R^2 = 0.082$, $p = 0.001$).

4. Discussion

To the best of our knowledge, there is no published work describing sDPPIV activity and its concentration in serum as being connected to anthropometric data, physical fitness, and physical activity together. Further, most published works about DDPIV and obesity show results related to concentration, not activity. The analysis of

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mRNA or protein levels of some myokines after exercise in tissue lysates or serum have shown discrepancies that confirm the poorly understood function of sDPPIV [8]. In previous works with cancer patients, the imbalance between activity and concentration has often been described. Thus, different progression patterns, not only for DPPIV, but also for other peptidases, have been described depending on the analyzed parameter (mRNA, protein concentration, or enzyme activity), the source of the sample (tissue or serum), or cancer type [28, 29]. Thus, it is not contradictory to find different relationships between sDPPIV and obesity depending on the analysis of concentration or activity.

DPPIV is linked to adiposity and obesity, but the regulation and extent of this connection remains unclear. In a cohort of healthy individuals, we measured their anthropometric characteristics and quantified several aspects of physical fitness and physical activity to determine whether these parameters were tied to DPPIV activity.

Our study has confirmed the close relationship between sDPPIV activity and obesity. Conflicting findings related to this association have been described [2, 3, 10, 11, 12, 13, 22]. Most authors have described a positive association between the amount of DPPIV protein present in the serum and obesity parameters [10, 12, 13]. Accordingly, some authors have reported the overexpression of DPPIV in visceral fat from adults with obesity and metabolic syndrome and an increase in DPPIV level along with the size of adipocytes in culture from obese adults [2]. However, sDPPIV levels have also been found to be inversely associated with waist circumference [22]. Our results agree with previous findings describing a negative correlation between DPPIV activity and increased body fat mass [3]. It is notable that our study, and others showing negative associations between obesity parameters and DPPIV, have been performed in apparently healthy populations [3, 22], while most studies showing positive correlations analyzed obese or diabetic subjects [10, 11, 12, 13]. In addition, the study by Neidert and coworkers [3] and the present research analyzed enzyme activity instead of protein concentration.

DPPIV is a 'moonlighting' glycosylated protein whose enzymatic activity can have a variety of effects. Its multifunctional activity depends on cell type and intracellular conditions that influence its role as a proteolytic enzyme, cell surface receptor, costimulatory interacting protein, or signal transduction mediator [5]. DPPIV degrades endomorphin, substance P, neuropeptide Y, insulin-like growth factor I, glucagon-like peptide 1 (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP), among other peptides [4, 7]. Based on its relationship with incretin hormones, previous work has related DPPIV to BMI, percent of fat mass, and other markers of obesity [13]. However, due to its multiple functions, it is not surprising that there are a variety of results describing DPPIV serum concentration and enzymatic activity [30].

Regarding physical fitness and taking into account the negative association between fitness and fatness [31], the positive association between the distance covered in

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6MWT and DPPIV agrees with the abovementioned negative link between DPPIV and obesity parameters. The 6MWT has been described as a simple, safe, and inexpensive sub-maximal exercise test [32], is a good marker of cardio respiratory fitness, and is suggested as a powerful predictor of survival for patients in clinical settings [33].

The number of CPMs is an indicator of total physical activity [34]. Thus, the positive association of the number of CPMs and time spent in light activity with sDPPIV activity allows us to conclude that higher rates of sDPPIV activity are correlated with more physical activity. Obesity and sedentary habits are indicators of cardiovascular risk [35], while the opposite is true for greater time spent in physical activity [36].

Variations in the concentration of sDPPIV linked to physical exercise and/or fitness could be related to the recently described auto/paracrine as well as endocrine effects of DPPIV as a myokine on organs such as liver, adipose tissue, and bone [37]. Based on our findings, sDPPIV activity is likely to be positively tied to exercise, and as a result, with cardiorespiratory and muscular health. This highlights the fact that exercise induces expression of some myokines but not all [37]. However, its role is far from clear as others have described a reduction in plasma DPPIV concentration after body mass loss or exercise training [11]. The different populations analyzed in both studies – apparently healthy individuals vs. subjects with metabolic syndrome – could be the reason for this discrepancy.

It is notable that there are sex-related differences in the interaction among adiposity, age, and cardiovascular risk, with the latter being more affected by age in women than in men. The activity of other peptidases related to cardiovascular risk has also been described previously as being dependent upon sex and age [21]. These results suggest that peptidases could be involved in the relationship between sex, age, and cardiovascular risk.

Several authors have described the lack of knowledge about the mechanism of DPPIV metabolism regulation *in vivo* [8]. Our results suggest that more complex analysis should be performed to clarify the role of DPPIV in obesity, physical fitness, and physical activity, as well as the pathways that regulate the balance between the enzymatically active and the inactive protein. Due to variations in DPPIV depending on cell type and intracellular or extracellular conditions that influence the role of DPPIV, it has been proposed as a therapeutic target in pathologic conditions such as diabetes [7].

In addition to the multifunctionality of DPPIV, other factors could explain the variation in studies relating this protein to obesity and health. Anthropometric characteristics, health status, ethnicity [3], lifestyle, and genetic characteristics could play a role in the relationship between BMI and DPPIV activity [1]. Several studies describe Asian obese patients [10, 38] and most have been carried out analyzing

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DPPIV concentration in obese, diabetic, or unhealthy individuals [2, 11]. Our work focused on sDPPIV activity in an apparently healthy Caucasian population and is in agreement with studies carried out in non-obese subjects. Nonetheless, the complex regulation pathways of DPPIV, the mechanism of its release from the plasma membrane, and its kinetics remain poorly understood, and further studies are needed to clarify the multiple functions and roles of DPPIV [2, 23].

The main limitation of this study is that due to its cross-sectional nature, causality cannot be inferred from our results. We have also to take into account that here presented results belonged to a healthy population; therefore these results cannot be directly extrapolated to obese people or individual pathologies. Another possible limitation of this study is that accelerometers do not capture exercises such as swimming and other aquatic sports. However, despite this limitation, the greater accuracy and precision of its use is well established in relation to the collection of data on physical activity through self-report questionnaires. In contrast, this study has important strengths. One is the bigger sample size than other studies analyzing the association between DPPIV and obesity. In addition, the majority of previous works describing DPPIV only presented the relationship between DPPIV and parameters related to obesity. However, in the present study, we also analyzed the relationship of DPPIV and physical fitness and physical activity measured in an objective way.

5. Conclusion

In conclusion, serum DPPIV activity not only depends on obesity parameters in healthy populations, it is also positively tied to some parameters of physical fitness and physical activity. In addition, sex and age related differences in sDPPIV activity have been found. Due to controversial results about the concentration of DPPIV and its enzymatic activity related to obesity and exercise, further investigations should seek to clarify the regulation and metabolism of this myokine with the purpose of enhancing its use as biomarker.

Declarations

Author contribution statement

Begoña Sanz: Performed the experiments; Wrote the paper.

Gorka Larrinaga: Performed the experiments; Analyzed and interpreted the data.

Javier Gil: Analyzed and interpreted the data.

Ainhoa Fernández-Atucha, Ana Belen Fraile-Bermudez, Maider Kortajarena, Andrea Izagirre: Performed the experiments.

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Pablo Martinez-Lage: Conceived and designed the experiments; Performed the experiments.

Jon Irazusta: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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