

Abdominal obesity as a risk factor for disability in Brazilian older adults

Ligiana Pires Corona^{1,*}, Tiago da Silva Alexandre², Yeda Aparecida de Oliveira Duarte³ and Maria Lúcia Lebrão⁴

¹School of Applied Sciences, University of Campinas, R. Pedro Zaccaria 1300, Limeira – SP, Brazil, 13484-350:

²Department of Gerontology, Universidade Federal de São Carlos, São Carlos – SP, Brazil: ³Department of Medical Surgical Nursing, School of Nursing, University of São Paulo, São Paulo – SP, Brazil: ⁴Department of Epidemiology, School of Public Health, University of São Paulo, São Paulo – SP, Brazil

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Abstract

Objective: To assess the role of abdominal obesity in the incidence of disability in older adults living in São Paulo, Brazil, in a 5-year period.

Design: Longitudinal study, part of the SABE Study (Health, Wellbeing and Aging). We assessed the disability incidence in the period (reported difficulty in at least one activity of daily living (ADL) in 2010) in relation to abdominal obesity in 2006 (waist circumference ≥ 102 cm in men and ≥ 88 cm in women). We used Poisson regression to evaluate the association between obesity and disability incidence, adjusting for sociodemographic and clinical factors including BMI.

Setting: São Paulo, Brazil.

Subjects: Older adults (n 1109) who were independent in ADL in 2006. In 2010, 789 of these were located and re-interviewed.

Results: The crude disability incidence (at least one ADL) was 27.1/1000 person-years in the period. The incidence rate was two times higher in participants with abdominal obesity compared with those without (39.1/1000 and 19.4/1000 person-years, respectively; $P < 0.001$). This pattern was observed in all BMI levels. In regression models, abdominal obesity remained associated with disability incidence (incidence rate ratio = 1.90; $P < 0.03$), even after controlling for BMI, gender, age, low grip strength, cognitive impairment, physical inactivity and chronic diseases.

Conclusions: Abdominal obesity was strong risk factor for disability, showing a more significant effect than BMI, and thus should be an intervention target for older adults. Waist measure is simple, cost-effective and easily interpreted, and therefore can be used in several settings to identify individuals at higher risk of disability.

Keywords
Obesity
Abdominal obesity
Disability
Older adults
SABE Study

With the rapid increase in the elderly population that has been observed in recent decades, functional disability is a major concern in relation to health and care demand. The presence of disabilities is associated with higher health-care needs and utilization compared with those with no disability, including recurrent hospitalization, greater use of outpatient care and increased risk of falls, injuries and acute illnesses^(1,2). This is an even greater concern in developing countries, since their demographic transition occurs faster and health services are not prepared to receive this growing demand⁽³⁾. Moreover, a rapid process of nutritional transition is ongoing in developing countries, with an increasing obesity and a still high proportion of underweight, which has a considerable impact on health-care services⁽⁴⁾.

Excess weight and obesity have been associated with a number of co-morbidities in all phases of life, especially chronic, non-transmittable conditions^(5,6). But in recent years, several studies have demonstrated that obesity is associated with limitations regarding physical function, independently of the presence of disease^(4,7–13). However, some results are not clear regarding obesity and disability. In a study carried out in the USA, class I obesity (BMI from 30 to 34.99 kg/m²) proved to be a protective factor against disability in men⁽¹⁴⁾. Nevertheless, several authors suggest that BMI high values are associated with increased bone mineral density and decreased osteoporosis and hip fracture in older men and women: the increase in bone mineral density and the extra cushioning around the trochanter (outer prominence of the femur) might provide

*Corresponding author: Email Ligiana.corona@fca.unicamp.br, lillypires@gmail.com

protection against hip fracture during a fall in obese older persons^(15–17), and thus could be protective against functional limitation after the fall.

The use of BMI is controversial in older adults. According to many authors, analysing only body weight may be a mistake, because changes in body composition may mask the real nutritional status, especially muscle wasting and fat distribution^(18–21). Moreover, several authors have pointed out that a BMI cut-off point of 25 kg/m² may be overly restrictive for older adults and have suggested that this threshold should be raised^(22,23). Some different, more conservative, cut-offs were proposed in the literature; the most known are the one proposed by Lipschitz⁽²³⁾, with an optimal range between 22 and 27 kg/m², and the one proposed by the American Committee on Diet and Health^(24,25), between 24 and 29 kg/m².

The main problem is that obesity should be defined as the amount of excessive fat storage and although BMI is a well-accepted surrogate of body fat, body composition changes may influence its use in older adults because both weight and height may be modified with ageing. The decrease in fat-free mass and increase in fat mass, combined with height loss caused by the compression of vertebral bodies, alter the relationship between BMI and percentage body fat. In addition, there is a higher relative increase in intra-abdominal fat than in subcutaneous or total body fat with ageing^(6,26,27).

Therefore, it is important to understand if the higher risk of disability can be due to weight in general or to a higher fat concentration. The second situation can be very common in older adults with 'normal' weight, considering those changes in body composition with ageing. For instance, a study in the adult population (20–79 years) of the National Health and Nutrition Examination Survey (NHANES) showed that about 30% of men and 69% of women with BMI in the normal range had abdominal obesity⁽²⁸⁾.

The quantification of visceral obesity is best determined by imaging examinations (such as computed tomography or dual-energy X-ray absorptiometry). The problem is that radiological measurements may not always be feasible in most clinical contexts, where anthropometric indicators are usually recommended^(6,29). Some recent studies have estimated adiposity using the waist circumference (WC) measure, which has been recommended as a better measurement for nutritional screening because it is easy to measure and strongly related to visceral and total fat^(30–32). WC is much more cost-effective and can be easily used at all health-care levels, especially in primary care, where resources may be scarce. In developing countries, like Brazil, it is important to count on cheap screening which can be effective in properly identifying people with higher risk.

Based on that, our hypothesis is that abdominal obesity can be a more informative measure and a risk marker independently from general obesity. Thus, the aim of the present study was to assess the role of abdominal obesity,

measured by WC, in the incidence of disability in older adults living in São Paulo, Brazil, in a 5-year period.

Methods

Sample and procedures

The data came from the SABE Study (Health, Wellbeing and Aging), which is a longitudinal study that began in 2000 and involved a multiple-stage probabilistic sample of individuals (aged 60 years or above) who live in São Paulo (*n* 2143). Individuals aged 75 years or above were over-sampled to compensate for the higher mortality rate in this age group. Sample weights took this oversample into consideration to represent the population. A second wave was carried out in 2006 and a third wave was conducted in 2010. In each new wave, a new sample of older adults between 60 and 64 years old was added following similar procedures used in the first wave. Details on the methodology of the study are described elsewhere^(33,34).

For the current analysis we used the second wave, carried out in 2006, as baseline (*n* 1413). We selected 1126 participants who were independent in activities of daily living (ADL). In 2010, 800 of them were located and re-interviewed. From the 1126 older adults selected at the baseline, seventeen did not have anthropometric measurements and were excluded. Thus, our final sample had 1109 independent older adults in 2006. Figure 1 describes the final sample.

Measurements

The 2006 and 2010 data included a household face-to-face interview conducted by a single interviewer using a standardized questionnaire addressing the living conditions and health status of the older adult respondent. Anthropometric measurements and physical tests were collected by a trained interviewer in another household visit.

The incident disability was the dependent variable and was recorded when the participant reported difficulty in one or more ADL in 2010 for which no difficulty was reported in 2006. The activities of daily living analysed were: walking across a room, dressing, bathing, feeding, transferring and toileting. Despite its importance among older adults, incontinence was not included because it does not necessarily imply physical limitation⁽³⁵⁾.

Body weight was measured using a calibrated scale, and height was measured using stadiometer fixed to a wall, with the barefoot individual wearing light clothing. BMI (kg/m²) was calculated by dividing body mass (in kilograms) by the square of height (in metres). Nutritional status was classified based on BMI cut-off points adopted by the Pan American Health Organization for the SABE Study⁽³⁶⁾: ≤ 23.0 kg/m² = underweight; > 23.0 and < 28.0 kg/m² = normal range (reference); ≥ 28.0 and < 30.0 kg/m² = overweight; ≥ 30.0 kg/m² = obesity. We adopted these cut-off points to

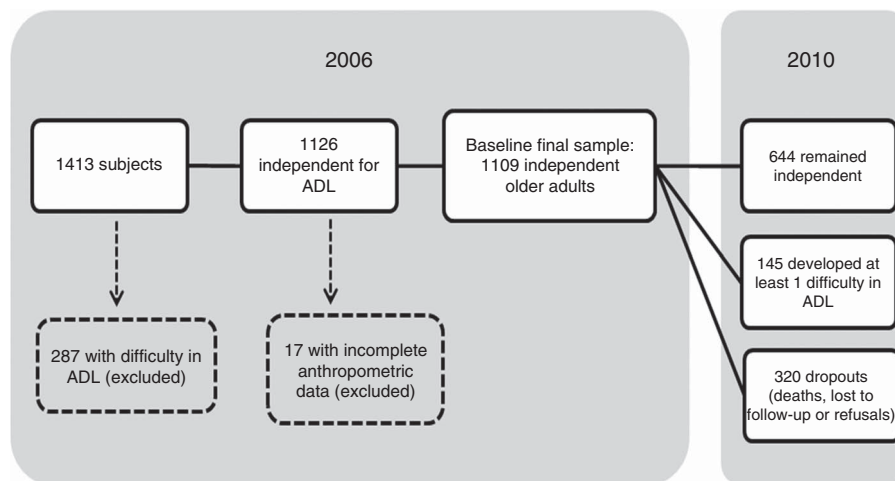


Fig. 1 Status of the SABE Study (Health, Wellbeing and Aging) sample, from 2006 baseline to the end of follow-up in 2010 (ADL, activities of daily living)

keep the same pattern of previous SABE publications and because they are really close to those proposed by Lipschitz⁽²³⁾ (i.e. the most used for older adults). Since obesity was less common than overweight and could compromise the statistical analysis due to the lower number of participants, we combined the two last categories, considering as overweight/obesity those with BMI values ≥ 28.0 kg/m².

Abdominal circumference was measured using an inelastic measure tape by a trained interviewer, at the midpoint between the last rib and the iliac crest, with the abdomen relaxed at the end of expiration, with the individual without shirt in an upright position with arms relaxed at body sides. Participants were classified with abdominal obesity when WC ≥ 102 cm in men and ≥ 88 cm in women, according to the cut-offs proposed by the National Cholesterol Education Program's Adult Treatment Panel III⁽³⁷⁾.

Sociodemographic and health-related variables measured at baseline were also included in the analysis. Baseline health status was assessed based on self-reported diabetes, hypertension, cancer, CVD, osteoarticular conditions, chronic respiratory disease and stroke.

Grip strength was measured using a dynamometer (Takei Kiki Kogyo® TK 1201), with two measurements, selecting the highest one between them. We classified low grip strength in men as values under 26 kg and in women as values under 16 kg⁽³⁸⁾.

Physical activity was self-reported using the Brazilian version of the International Physical Activity Questionnaire⁽³⁹⁾. We classified as physically inactive (sedentary) those who reported less than 150 min of moderate activities per week or less than 75 min of vigorous activities per week⁽⁴⁰⁾.

Cognitive status was evaluated using a modified version of the Mini Mental State Exam validated for the SABE Study, due to the low level of schooling of the South American older adult population. This measurement has

thirteen items that are less dependent upon schooling, with a total possible score of 19 points. Those with a score of 12 or lower were classified as having cognitive impairment⁽⁴¹⁾.

Statistical analysis

For the descriptive analysis, mean values and their standard errors were calculated for continuous variables, and proportions were calculated for categorical variables. Differences between groups were estimated using the Wald test of mean equality and Rao–Scott correction, which considers sample weights for estimates with population weights.

The crude density of the incidence of disability in the 5-year period was calculated considering the participants who did not have disability at baseline. For the calculation of incidence density, the numerator was made up of the number of individuals who developed difficulty in one or more ADL in the period and the observation times were summed in the denominator. In cases of death, the observation time was the interval between the 2006 interview and the date of death. For deaths with unknown date, the observation time was the interval between the 2006 interview and a date attributed to the death based on the mean date of death of known cases in the same age group and gender. For those who did not develop disabilities, the observation time was the period between the 2006 interview and 2010 interview. For those who developed disabilities, the observation time was half the period between the 2006 and 2010 interviews. Refusals to participate, cases of institutionalization and non-located individuals also counted for half the period between the 2006 and 2010 interviews, once it was not possible to determine the date. The incidence density was also calculated according to BMI categories and for each ADL separately, according to abdominal obesity.

Poisson multiple regression analysis was performed to evaluate the association between abdominal obesity and disability incidence in the period, incorporating those covariables with a P value <0.20 in the univariate regression. Variables were maintained in the final model if statistically significant ($P < 0.05$) or if they adjusted the estimates by at least 10%. All analyses included sample weights and were adjusted for the complex sampling design. Data analyses were performed using the statistical software package Stata[®] version 12.

Results

Table 1 shows the characteristics of the participants at baseline, according to their follow-up status. Among the 1109 independent participants at baseline, mean age was 69.2 years, schooling average was 4.5 years, mean BMI was 26.6 kg/m² and mean WC was 90.8 cm. After the 5-year period, 146 participants died and 174 refused to participate or were lost in the period. Most of the dropouts were men, older, had lower BMI and grip strength values, lower prevalence of abdominal obesity and higher prevalence of cognitive impairment. Education, physical inactivity and self-reported chronic conditions did not differ.

Among the 789 older adults interviewed in 2010, 644 remained independent and 145 developed at least one

difficulty in ADL. Participants with incident disability were older, less educated and had higher prevalence of chronic conditions at baseline, except for stroke, cardiovascular and pulmonary disease, which were not significantly associated with disability incidence. Nutritional and physical statuses at baseline were also different between groups: those who had incident disability had higher BMI and WC, lower hand grip strength, and higher proportions of obesity and abdominal obesity.

The crude incidence rate of ADL disability (at least one ADL) for all participants was 27.1 per 1000 person-years in the period. The incidence rate was two times higher for those with abdominal obesity in 2006 compared with those without it (39.1 and 19.4/1000 person-years, respectively; $P < 0.001$). This pattern was observed in all BMI levels (Table 2), including normal and underweight older adults.

Table 2 also presents the incidence rate of each ADL. The activities with higher incidence were dressing, toileting and bathing, while feeding was less incident. Older adults with abdominal obesity had higher incidence in all activities, particularly in dressing and toileting, in which the rate was more than double relative to those without abdominal obesity ($P < 0.001$).

Table 3 displays the results of Poisson regression models for the incidence of disability. In the crude models, general obesity and abdominal obesity were both

Table 1 Characteristics at baseline and after the follow-up period, according to outcome, among the sample of older adults (≥ 60 years old) in São Paulo, Brazil. SABE Study (Health, Wellbeing and Aging), 2006 and 2010

Baseline characteristic	Total sample in 2006 (baseline) (n 1109)		Dropouts (died/lost to follow-up) (n 320)		Follow-up				P value*
	Mean	95% CI	Mean	95% CI	Independent in 2010 (n 644)		Dependent in 2010 (n 145)		
Gender (%)									0.039
Male		43.0		53.0		89.0		11.0	
Female		57.0		47.0		84.3		15.7	
Age (years)	69.2	68.1, 70.3	70.7	69.1, 72.2	68.2	67.1, 69.2	72.4	70.7, 74.1	0.001
Years of schooling	4.5	4.0, 5.1	4.4	3.8, 4.9	4.8	4.2, 5.4	3.4	2.8, 4.0	<0.001
BMI (kg/m ²)	26.6	26.3, 27.0	25.8	25.2, 26.4	26.8	26.3, 27.2	28.1	26.9, 29.3	0.055
Normal weight (%)		44.3		45.7		45.5		33.2	
Underweight (%)		21.8		28.3		19.3		21.3	0.151
Overweight/obesity (%)		33.9		26.0		35.2		45.5	0.018
Waist circumference (cm)	90.8	89.8, 91.8	89.8	88.0, 91.5	90.7	89.6, 91.9	93.8	91.1, 96.4	0.041
Abdominal obesity (%)		39.2		32.6		39.1		56.4	0.003
Grip strength (kg)	25.7	24.9, 26.4	25.6	24.1, 27.0	26.4	25.6, 27.2	21.3	19.7, 23.0	<0.001
Low grip strength (%)		19.7		25.6		14.8		35.8	<0.001
Physical inactivity/sedentary (%)		60.7		63.4		58.9		72.1	0.044
Self-reported chronic conditions (%)									
Hypertension		59.9		56.0		59.2		74.4	0.001
Diabetes		19.3		21.9		16.6		27.6	0.010
Cancer		4.2		6.0		3.0		7.6	0.024
Chronic pulmonary disease		10.6		13.3		9.5		10.5	0.781
CVD		20.4		21.8		19.1		25.7	0.087
Stroke		5.8		7.9		4.3		10.2	0.070
Osteoarthritis		29.6		23.2		29.7		44.9	0.007
Osteoporosis		19.4		16.2		19.2		28.2	0.019
Cognitive impairment (%)		7.8		11.9		4.8		16.3	<0.001

Rao-Scott and Wald tests were used to assess differences.

*P value for comparison between dependent and independent in 2010.

Table 2 Incidence rate of disability in activities of daily living per 1000 person-years in older adults (≥ 60 years old) in São Paulo, Brazil, according to the presence of abdominal obesity and BMI categories. SABE Study (Health, Wellbeing and Aging), 2006–2010

	Total	95 % CI	No abdominal obesity	95 % CI	Abdominal obesity	95 % CI
At least one of the activities, by BMI category						
All participants	27.1	22.4, 33.0	19.4	14.7, 26.2	39.1	30.3, 51.1
Normal weight	20.1	14.6, 28.6	16.2	10.8, 25.6	33.1	19.6, 59.7
Underweight	27.8	18.5, 43.3	27.0	17.9, 42.5	49.1	31.4, 60.1
Overweight/obesity	36.1	27.2, 48.7	13.6	4.9, 51.3	41.0	30.6, 56.0
By type of activity, all participants						
Walking across a room	8.0	5.7, 11.6	6.5	3.9, 11.7	10.4	6.72, 16.8
Dressing	15.3	12.1, 19.6	10.3	7.3, 15.1	23.0	16.7, 32.4
Bathing	11.2	8.6, 14.8	9.4	6.6, 13.6	14.0	9.3, 21.8
Feeding	3.9	2.5, 6.4	3.8	2.1, 7.4	4.1	2.1, 9.3
Transferring	10.0	7.5, 13.6	7.9	5.1, 12.9	13.3	9.1, 20.1
Toileting	12.8	9.9, 16.9	8.9	6.1, 13.7	18.8	13.3, 27.5

Table 3 Results of Poisson regression models for disability incidence in older adults (≥ 60 years old) in São Paulo, Brazil. SABE Study (Health, Wellbeing and Aging), 2006–2010

	Unadjusted IRR		Adjusted IRR*	
	IRR	P value	IRR	P value
Abdominal obesity BMI	1.82	0.003	1.90	0.031
Normal weight	1.00	–	1.00	–
Underweight	1.43	0.151	1.67	0.032
Overweight/obesity	1.64	0.018	1.18	0.535
Age	1.08	<0.001	1.06	<0.001
Gender, female	1.43	0.042	0.94	0.712
Years of education	0.91	<0.001	0.94	0.022
Low grip strength	2.60	<0.001	1.65	0.022
Physical inactivity	1.68	0.051	1.44	0.205
Hypertension	1.84	0.013	1.16	0.536
Diabetes	1.72	0.009	1.33	0.188
Cancer	2.02	0.017	1.69	0.097
Osteoarthritis	1.75	0.007	1.61	0.008
Osteoporosis	1.53	0.019	1.11	0.586
Cognitive impairment	2.85	<0.001	1.46	0.123

IRR, incidence rate ratio.

*Adjusted for age, female gender, years of schooling, low grip strength, physical inactivity, self-reported chronic conditions (hypertension, diabetes, cancer, osteoarthritis, osteoporosis) and cognitive impairment.

predictive of disability. When both variables were in the same model, controlled for the other significant factors presented in the descriptive analysis, abdominal obesity was still significant, presenting a risk 1.90 higher than for those without abdominal obesity ($P < 0.03$), and general overweight/obesity represented by BMI was no longer significant.

Discussion

Our results showed that abdominal obesity is a risk factor for disability in older adults, independent from obesity. An association between obesity and disability is already well documented in the literature^(6–8,14,42–44). In Brazil, our group had already shown that obesity was associated with

higher incidence of instrumental ADL disability in a 6-year period⁽⁴⁾, as well as ADL limitations and lower recovery from Nagi's limitations⁽⁴⁵⁾.

A meta-analysis conducted by Carmienke *et al.*⁽⁴⁶⁾ reported that when both BMI and WC measurements were included in the same model, BMI showed either a negative significant or an insignificant association with all-cause mortality, whereas WC showed a significant positive association with mortality when controlled for BMI. The authors recommended that abdominal obesity measurements should be used in clinical practice, in addition to BMI, to assess obesity-related mortality in adults.

Nevertheless, there are few longitudinal studies with community-living older adults, and some of them have conflicting results. A prospective study in Spain showed that the higher quintile of WC was associated with higher incidence of mobility difficulties, but not with ADL disability⁽⁴⁷⁾. Visser *et al.*⁽⁴⁸⁾ did not find a significant association between WC and disability in older participants from the Framingham Study (but the data were not shown in the paper). However, Chen and Guo⁽⁴⁹⁾ analysed the NHANES population in the USA and found that both BMI and WC were predictive for disability in several degrees; but when in the same model, WC attenuated most of the BMI effects. Chen *et al.*⁽⁵⁰⁾ had already shown the same effect in Hispanic older adults: when BMI and WC were included in the same model, WC, but not BMI, remained significantly associated with disability. Na *et al.*⁽⁵¹⁾ also found that BMI was not related to disability in Korean older adults and abdominal obesity increased the odds of ADL limitation by 2.7-fold.

Studies analysing abdominal obesity and disability are rare in developing countries. In Brazil, so far studies are cross-sectional and have small samples. Nevertheless, they already showed some associations. Campanha-Versiani *et al.*⁽⁵²⁾ conducted a cross-sectional study with forty-eight women and showed that abdominal obesity was associated with lower scores in functional tests. Another

cross-sectional study with seventy-seven older adults showed that abdominal circumference was higher in frail individuals⁽⁵³⁾. The only study with a larger sample was a FIBRA (Frailty in Brazilian Elderly) cross-sectional multi-centre study that showed that a higher WC was associated with frailty in all BMI categories⁽⁵⁴⁾.

Among the possible mechanisms to explain our findings, the most cited in the literature is that obesity can represent a 'burden' for the osteomuscular system and can also increase postural instability, which can lead to higher risk of falls (particularly in abdominally obese individuals)⁽⁵⁵⁾ and limit activities^(12,47,56,57). Several authors also point out that, due to the fact that abdominal obesity is highly associated with diseases that lead to disability (such as diabetes, cardiovascular conditions, cancer, etc.), this association could be confounded^(47,51,56,58); however, our results are consistent even after controlling most of those conditions, including osteoarthritis.

This higher burden that obesity could cause to the osteomuscular system may not be clear enough if only body weight is analysed; many studies have concluded that obesity was associated with higher bone mineral density and could actually prevent fractures^(15–17). But more recently the literature has been demonstrating that obesity induces chronic inflammation, which may reduce muscle mass and increase bone absorption^(27,51,59), and thus obesity could even increase risk of ankle and upper leg fractures⁽⁶⁰⁾. Inflammation is already known as an important risk factor for disability, mainly mediated by its role in sarcopenia and lower physical function^(59,61–63). In this sense, it is plausible that abdominal obesity may have a role in disability modulated by inflammation, independently of chronic diseases.

Another point that should be considered is that weight loss has been widely described as a risk factor for mortality. Several studies have already shown that weight change (weight loss or weight gain) is more harmful than maintaining weight during the ageing process^(64–67). So maybe general obesity should not be targeted with severe weight-loss programmes, but abdominal obesity should be targeted to reduce risk, instead of body weight *per se*.

It is important to notice that, when analysing each ADL, the incidence for dressing and toileting were higher. In a study analysing SABE's first follow-up period (2000 to 2006), the most incident ADL were dressing and transferring for men and women; toileting had an incidence of 4.3/1000 persons per year for men and 10.9/1000 persons per year for women⁽⁶⁸⁾. Jagger *et al.*⁽⁶⁹⁾, on the other hand, found that women had higher risk of disability in bathing and toileting relative to men, but the order of activity restriction was bathing, mobility, toileting, dressing, transfers from bed and chair, and feeding. They suggested that lower-extremity disability (bathing, mobility, toileting) precedes upper-extremity disability (feeding), with difficulty for dressing being either upper-extremity or lower-extremity disability⁽⁶⁹⁾. Dunlop *et al.*⁽⁷⁰⁾, on the contrary, argued that

dressing may require only upper-extremity flexibility/dexterity, in addition to cognitive functioning. In our point of view, dressing requires upper-limb strength, fine motor coordination, flexibility, lower-limb strength and balance⁽⁶⁸⁾, and these conditions can be strongly affected by abdominal obesity^(54,71,72).

In our study, the incidence was more than two times higher in abdominally obese older adults for the activities of both dressing and toileting. Guallar-Castillón *et al.*⁽⁴⁷⁾ also found that the highest quintile of WC was associated with bathing or dressing difficulties in women, but not in men. So, it is plausible to understand that dressing could be affected by a 'physical barrier' such as a higher WC to cope with all those skills. The same explanation can be hypothesized for toileting in our study: the physical barrier can impact skills that can be necessary to perform this activity without help, such as flexibility, strength and mobility, and balance.

Our results should be interpreted taking some points into consideration. Disability was measured using self-reported information; nevertheless, methodological studies have demonstrated that self-reported data on functional disability have adequate validity and are consistent with medical diagnoses and/or physical tests⁽⁷³⁾. Another limitation is the high proportion of losses, which may influence our results.

The study also has some strong points. First, it was conducted in a large representative sample of community-living older adults in the biggest city in Brazil. Second, it is a prospective cohort that analysed only independent older adults at baseline, so it shows a possible role of abdominal obesity in the disability pathway. To our knowledge, the present study is the first one in Brazil with a large representative sample and with longitudinal follow-up.

Conclusion

Our study shows that abdominal obesity is a risk factor for disability in older adults, independently from BMI, and we consider that WC is a simple, cost-effective and easily interpreted measurement, and therefore can be used in several settings, from hospitals to primary care facilities, to identify individuals with higher risk of disability. Thus, abdominal obesity should be a target for intervention to avoid or postpone disability, cardiovascular events and mortality.

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responsible for study design and formulated the research question. Y.A.O.D. and M.L.L. were responsible for data collection and are Principal Investigators of the main study. The statistical analyses, results and discussion were performed by L.P.C. and T.S.A. All authors participated in the drafting of the manuscript and approved its final version. *Ethics of human subject participation:* This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Research Ethics Committee at the University of São Paulo. Written informed consent was obtained from all subjects/patients.

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