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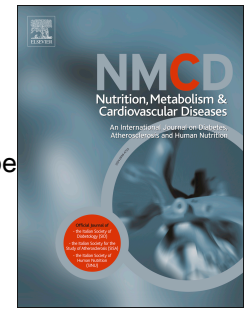

Association of free-living physical activity measures with metabolic phenotypes in type 2 diabetes at the time of diagnosis. The Verona Newly Diagnosed Type 2 Diabetes Study (VNDS)

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Title: Association of free-living physical activity measures with metabolic phenotypes in type 2 diabetes at the time of diagnosis. The Verona Newly Diagnosed Type 2 Diabetes Study (VNDS).

Running title: Physical activity in newly-diagnosed type 2 diabetes.

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Abstract

Objective – Lifestyle is considered a major determinant of risk of type 2 diabetes (T2D). We investigated whether daily physical activity (DPA) is associated with beta-cell function (BF) and/or insulin sensitivity (IS) in patients with T2D at the time of diagnosis.

Methods – In 41 subjects enrolled in the Verona Newly-Diagnosed Type 2 Diabetes Study we assessed: (1) IS, by euglycaemic insulin clamp; (2) BF, estimated by prolonged-OGTT minimal modeling and expressed as derivative and proportional control; (3) DPA and energy expenditure (EE), assessed over 48-hours monitoring by a validated wearable armband system.

Results – Study participants (median[IQR]; age: 62 [53-67] years, BMI: 30.8 [26.5-34.3] $Kg \cdot m^{-2}$, HbA1c: 6.7 [6.3-7.3]%; 49.7 [45.4-56.3] mmol/mol) were moderately active (footsteps/day: 7,773 [5,748-10,927]; $DPA_{\geq 3MET}$: 70 [38-125] min/day), but none of them exercised above 6 metabolic equivalents (MET). EE, expressed as EE_{TOT} (total daily-EE) and $EE_{\geq 3MET}$ (EE due to $DPA_{\geq 3MET}$) were 2,398 [2,226-2,801] and 364 [238-617] Kcal/day, respectively. IS (M-clamp 630 [371-878] $\mu mol/min/m^2$) was positively associated with DPA and EE, independent of age, sex and BMI ($p<0.05$). Among the DPA and EE parameters assessed, $DPA_{\geq 3MET}$ and EE_{TOT} were independent predictors of IS in multivariable regression analyses, adjusted for age, sex, BMI (R^2=16%, R^2=19%, respectively; $p<0.01$). None of model-derived components of BF was significantly associated with DPA or accompanying EE.

Conclusions – Our study highlighted moderate levels of DPA and total EE as potential determinants of IS, but not BF, in T2D at the time of diagnosis. Intervention studies are needed to conclusively elucidate the effect of DPA on these features.

Clinical Trial Registration – URL: http://www.clinicaltrials.gov; Unique Identifier: NCT01526720

Keywords

Type 2 diabetes; beta-cell function; insulin sensitivity; physical activity; energy expenditure

Abbreviations

T2D = type 2 diabetes

VNDS = the Verona Newly-Diagnosed Type 2 Diabetes Study

IS = insulin sensitivity

BF = beta-cell function

OGTT = oral glucose tolerance test

EE = energy expenditure

DPA = daily physical activity

MET = metabolic equivalent

$DPA_{\geq 3MET}$ = physical activity ≥3 MET

EE_{TOT} = total daily energy expenditure

$EE_{\geq 3MET}$ = energy expenditure due to $DPA_{\geq 3MET}$

BMI = body mass index

BSA = body surface area

GAD-antibodies = Glutamic Acid Decarboxylase Autoantibodies

ISR = insulin secretion rate

DC = derivative control of beta-cell function

PC = proportional control of beta-cell function

SWA = SenseWear® Armband

INTRODUCTION

Type 2 diabetes (T2D) is characterized by a combination of defective beta-cell function (BF) and impaired insulin sensitivity (IS) [1], and frequently occurs as a consequence of sedentary lifestyle and “westernized” dietary habits [2, 3]. Alarming global projections for diabetes and obesity epidemics claim prompt multidisciplinary interventions and better prevention strategies to effectively target the complexity of T2D landscape [4]. Among the therapeutic lifestyle changes, a general increase in daily physical activity (DPA) is considered of pivotal relevance among the recommended interventions of current structured programs for diabetes prevention and care [5, 6]. However, notwithstanding the large evidence provided by the Diabetes Prevention Program (DPP) and other trials [7, 8] supporting the benefits of higher DPA on T2D risk, the relationship between DPA during free-living conditions and the pathophysiologic determinants of T2D (i.e. defective BF and impaired IS) at the time of disease diagnosis is still imperfectly known.

Recently, Solomon *et al.* reported a linear association between lower IS and poorer cardiorespiratory fitness, a measure of physical activity, evaluated by gold-standard incremental stress test across the entire spectrum of glucose tolerance [9]. The authors also showed a positive relationship between cardiorespiratory fitness and the disposition index, a measure of insulin secretory compensation accounting for changing in IS. Interestingly, with regard to insulin secretion alone, considered irrespectively of extant IS, the authors identified a robust and negative association of cardiorespiratory fitness with both early and late phase of insulin secretion during an oral glucose tolerance test (OGTT) in individuals with normal and impaired glucose tolerance [9]. However, the association was considerably weaker (early phase: $r=-0.23$, $p<0.05$) or absent (second phase) in T2D patients. This might reflect the peculiar effect of exercise on the pathophysiologic determinants of T2D once the disease has established, and, as such, it warrants further investigation.

Additionally, while there is a relatively large evidence supporting the beneficial effects of physical activity - alone, or as part of more structured lifestyle interventions - on IS [10-13],

only a limited number of studies have reported on the physical activity of T2D patients during free-living conditions using objective measures [14, 15], and no literature is available in patients with newly-diagnosed T2D. Indeed, while accumulating evidence shows that intensive exercise training improves both BF and IS [16], there are only scanty data on the effect of moderate levels of physical activity on these aspects [17].

Therefore, in the present study we explored the amount of free-living DPA and accompanying energy expenditure (EE), assessed by a wearable motion sensor, in a sample of drug-naïve patients with newly-diagnosed T2D enrolled in the Verona Newly Diagnosed Type 2 Diabetes Study (VNDS). In particular, we investigated the association of DPA and EE with state-of-the-art assessed BF and IS.

RESEARCH DESIGN AND METHODS

Study population

The Verona Newly Diagnosed Type 2 Diabetes Study (VNDS) is an ongoing research project, aimed at building a bio-bank of patients of Italian ancestry with GAD-antibodies negative (GAD65 <1 KU/L), drug-naïve, newly-diagnosed diabetes. A detailed description of the study protocol has been specified elsewhere [18] and is also available in the **Supplementary Material**. The registered study protocol is available online at https://clinicaltrials.gov (ClinicalTrials.gov Identifier: NCT01526720).

A standardized medical interview was conducted in each individual, alongside with a detailed physical examination, screening for chronic complications, assessment of body composition (% fat mass) by electrical bioimpedance (Tanita SC 330P, Tanita Corporation of America, Inc, Arlington Heights, 60005 IL, USA) and laboratory measurements, including those detailed in **Table I**. All study participants were tested on two separate days and in random order with a 75-grams prolonged (5-hours) oral glucose tolerance test (OGTT) to assess beta-cell function (BF), and with a hyperinsulinemic euglycaemic clamp to assess insulin sensitivity (IS), as detailed below. The study participants were drug-naïve or, if already treated with antidiabetic drugs, underwent a treatment washout of at least one week before performing metabolic tests. All subjects consumed a weight-maintaining diet containing 200-250 g of carbohydrate/day for at least three days before studies and no subject participated in any heavy exercise. Body weight was stable in all subjects for at least 1 month before metabolic tests. The project is also backbone for *ad hoc* ancillary sub-projects or pilot studies according to scientific questions to be specifically addressed. In this paper we report data on daily physical activity (DPA) and accompanying energy expenditure (EE) recorded in 41 random VNDS participants during 48 hours of free-living conditions within the first week after the metabolic tests. The anthropometric, clinical and metabolic features of the study participants are summarized in **Table I**. None of the study subjects

exercised above 6 METs; therefore, only data of EE and DPA pertaining to light-to-moderate exercise levels are presented and discussed here.

The VNDS study protocol was approved by the local Institutional Review Board, and all subjects gave written informed consent upon recruitment.

Standard biochemistry

Plasma glucose concentration was measured in duplicate with a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA, USA) or an YSI 2300 Stat Plus Glucose&Lactate Analyzer (YSI Inc., Yellow Springs, OH, USA), at bedside. Serum C-peptide and insulin concentrations were measured by chemiluminescence [19]. Glycosylated hemoglobin (HbA1c) was measured by a high-performance liquid chromatography analyzer on Tosoh G7 automated analyzer (Tosoh Bioscience Inc., San Francisco, CA; USA); the upper limit of normal for our laboratory was 5.6% (38 mmol/mol). Serum lipids were assessed by standard in-house methods. GAD-antibodies were measured by immunoradiometry (CentAK, Medipan, Germany), according to manufacturer's instructions.

Assessment of beta-cell function

The analysis of the glucose and C-peptide time courses during OGTT was performed by mathematical modeling as previously described [18]. The insulin secretion rate (ISR) was modeled as comprised by two components, one being function of the rate of glucose increase and the other dependent on glucose concentration *per se*. Model estimates of both ISR components were normalized by body surface area (BSA) and were calculated to obtain two main physiological outputs, as follows:

1. Derivative control (DC) of BF, expressed as the amount of insulin secreted in response to a rate of glucose increase of 1 mmol/L/min, which lasts for 1 min [ISR, $(pmol/m^2_{BSA})\ (mmol/L/min)^{-1}$];
2. Proportional control (PC) of BF, representing the stimulus-response curve linking the glucose concentration (*x*-axis) to the insulin secretion rate [ISR, $(pmol/min/m^2_{BSA})$]

at preselected glucose concentrations of 5.5, 8.0, 11.0, 15.0 and 20.0 mmol/L.

Further modeling details are available in the **Supplementary Material**.

Assessment of insulin sensitivity

On a separate day, as detailed in the **Supplementary Material** and in prior publications [20-22], the individual insulin sensitivity (IS) of each study participant was assessed by applying the gold-standard technique of the hyperinsulinemic euglycaemic clamp [23, 24], and it was expressed as the amount of glucose metabolized during the last 60 min of the clamp.

Assessment of physical activity and energy expenditure

Individual data on physical activity and energy expenditure were recorded over 48 hours monitoring and expressed as a daily average. Physical activity was classified according to the corresponding energy cost (also dubbed as MET, metabolic equivalent, according to the definition of Jetté et al. [25]) as light (<3 METs), moderate (3≤ METs <6) or intense (≥6 METs) and it was either expressed as the number of footsteps per day or as exercise duration (minutes per day) in each MET category.

Daily physical activity (DPA), total daily energy expenditure (EE_{TOT}, kilocalories per day) and energy expenditure due to DPA ≥3 MET ($EE_{\geq 3MET}$) were measured by the SenseWear® Armband (SWA, Body Media Inc, Pittsburgh, PA).

The SWA is a wearable armband inertial sensor previously validated as effective and reliable instrument to assess free-living physical activity and EE [26, 27]. The device is comprised of a built-in two-axis accelerometer, a heatflux and skin temperature sensor, a galvanic skin response sensor, and a near-body ambient temperature sensor to obtain individual data. The SWA was randomly offered to patients upon enrollment in the VNDS study. It was positioned over the triceps muscle on the upper right arm and kept in place for two consecutive days. All participants were instructed to remove the armband only for bathing purposes.

Statistical Analysis

Data are presented as means ±SD or median and interquartile range [IQR], unless otherwise indicated. Before comparisons, skewed variables were natural log-transformed to correct for non-Gaussian distributions. Unpaired Student's *t* test or chi-square test was applied for continuous or categorical variables, respectively, to test for sex-related differences in the clinical, anthropometric or metabolic characteristics among the study participants. The bivariate association (expressed as Pearson's correlation coefficient, with significance set at two-tailed *p*-value <0.05) of physical activity measures with body composition and metabolic parameters was explored first in the whole sample and thereafter separately in men and women. One-way ANOVA, adjusted for age, sex and body-mass index (BMI), was carried out to explore the distribution of insulin sensitivity across incremental categories of physical activity and energy expenditure measures, calculated as tertiles of the daily number of footsteps, $DPA_{\geq 3MET}$, EE_{TOT} and $EE_{\geq 3MET}$. The same analyses were applied to women and men, respectively.

Generalized linear regression models (GLM) were applied to evaluate the contribution to IS variance (dependent variable) carried by the daily number of footsteps (Model M1), $DPA_{\geq 3MET}$ (Model M2), EE_{TOT} (Model M3) or $EE_{\geq 3MET}$ (Model M4), independent of age, sex and BMI. The same analyses were conducted by replacing BMI with waist circumference. The covariates included in the multivariable regression analyses were chosen as potential confounding factors on the basis of their significance in univariate analysis or on their biological plausibility. A stepwise-forward linear regression model including footsteps/day, $DPA_{\geq 3MET}$, $EE_{\geq 3MET}$, EE_{TOT}, age, sex, BMI and waist as potential predictors of IS was applied to account for possible collinearity issues. All statistical analyses were carried out with SPSS 22.0 software. Type I error rate was set at two-tailed $p<0.05$.

RESULTS

As shown in **Table I**, the study sample was mainly comprised of middle-aged, overweight T2D patients in a fairly good overall glycometabolic control. According to the study protocol,

none of them was on oral hypoglycemic agents or on insulin therapy, while 24.3, 60.9 and 24.4% was on lipid-lowering, anti-hypertensive and/or aspirin medication, respectively. The duration of $DPA_{\geq 3MET}$ and daily EE (either total or ≥ 3 METs) were higher in males. No further sex differences were found in the other parameters assessed.

In bivariate analysis, DPA (footsteps and $DPA_{\geq 3MET}$) and EE (EE_{TOT} and $EE_{\geq 3MET}$) were associated with several body composition measures and metabolic parameters, but not with model-derived BF metrics (**Table II** and **Supplementary Figures S1, S2, S5-S9**). In particular, both footsteps and $DPA_{\geq 3MET}$ showed a significant, inverse association with BMI, waist circumference, and triglyceride levels, while bioimpendance-assessed fat-mass was inversely associated with $DPA_{\geq 3MET}$ only. Higher levels of $DPA_{\geq 3MET}$, $EE_{\geq 3MET}$ and incremental footsteps/day were also significantly associated with higher HDL-cholesterol levels and higher clamp-assessed IS, particularly in men.

As shown in **Figure 1**, when IS was considered as a categorical variable and expressed as tertiles of M-clamp, the distribution of $DPA_{\geq 3MET}$, EE_{TOT}, $EE_{\geq 3MET}$ and footsteps across incremental categories of IS was robust against correction for age, sex and BMI (one-way ANOVA, $p<0.05$, **Figure 1**). Similar results were also obtained by replacement of BMI with either waist or bioimpedance-assessed fat-mass (data not shown). Separate analyses by sex (**Figure S3**-**S4**) showed similar trends in the distribution of physical activity and energy expenditure measures, although statistical significance was reached only for $DPA_{\geq 3MET}$ in men, probably due to limited statistical power.

In GLMs adjusted for age, sex and BMI (model M2: R^2=22%, p=0.05; model M3: R^2=24%, p=0.03, **Table III**), $DPA_{\geq 3MET}$ and EE_{TOT} were significantly associated with IS, each explaining 16% and 19% of IS variance, respectively. Similar results were obtained in analogous GLMs (models M1, M4) for the number of footsteps/day and $EE_{\geq 3MET}$, although both models underperformed in their overall descriptive ability of IS variance (M1: R^2=18%, p=0.11; M4: R^2=21%, p=0.08).

In GLMs adjusted for age, sex and waist circumference (model M2: R^2=24.2%, p=0.04; model M3: R^2=35%, p=0.003; model M4: R^2=24%, p=0.04, **Table IV**), $DPA_{\geq 3MET}$, EE_{TOT} and

$EE_{\geq 3MET}$ were significantly associated with IS, each explaining 16%, 8% and 14% of IS variance, respectively. The model including the number of footsteps/day did not perform sufficiently to describe the IS variance (M1: R^2=21.3%, p=0.07).

Eventually, only $DPA_{\geq 3MET}$ survived as independent predictor of IS (R^2=16%, p<0.009), when entered in a stepwise-forward linear regression model also including $EE_{\geq 3MET}$, the number of footsteps/day, EE_{TOT}, age, sex, BMI and waist.

DISCUSSION

In this analysis, conducted in a random subset of adults with newly-diagnosed T2D participating in the VNDS Study, we observed that: 1) higher levels of free-living DPA and accompanying EE were consistently associated with several body composition measures and metabolic parameters in the expected effect direction (namely, higher IS, lower adiposity measures and a more favorable lipid profile); 2) the positive association of IS with incremental DPA and EE was robust against correction for age, sex and adiposity measures; 3) among the DPA and EE parameters assessed, $DPA_{\geq 3MET}$, EE_{TOT} and $EE_{\geq 3MET}$ individually resulted as significant predictors of IS independently of age, sex and adiposity measures; 4) only $DPA_{\geq 3MET}$ eventually survived as independent predictor of IS variance in a comprehensive stepwise-forward linear regression model also including $EE_{\geq 3MET}$, the number of footsteps/day, EE_{TOT}, age, sex, BMI and waist); 5) DPA and EE were not significantly associated with the model-derived measures of BF.

Our findings represent, to our knowledge, the first report of DPA and accompanying EE in patients with newly-diagnosed T2D. They are in line with previous observations regarding the association of moderate-to-vigorous physical activity with a more favorable cardio-metabolic profile in both T2D patients [15] and in adult individuals from the general population [28]. Interestingly, we herein reported the first evidence of a strong and positive association of incremental levels of DPA and EE with whole-body IS in free-living conditions, by assessing BF and IS with state-of-the-art techniques.

The underlying mechanisms as to why DPA, EE and footsteps may be associated with IS could not be explored in detail in the present study, as we did not employ tracers or hormonal assays to dissect the complex interplay between IS and BF by quantitatively estimating the changes of hepatic gluconeogenesis and the glucose uptake by the skeletal muscle and the adipose tissue. We rather identified $DPA_{\geq 3MET}$ as the principal and independent determinant of IS by a multiple-step regression approach, which allowed to account for the high concordance existing among the measures of physical activity and

energy expenditure assessed by the armband system. The separate analyses conducted to clarify the predictors of IS according to different measures of adiposity revealed a better performance of waist circumference, as compared to BMI, at improving the overall descriptive ability of the regression models. This points to the relevance of fat distribution, rather than general adiposity, in the overall economy of body energy balance. Of note, the association with higher IS was limited to $DPA_{\geq 3MET}$, while the number of footsteps/day (which could be considered a reasonable proxy for the total volume of DPA in our patients) did not play a significant contribution to IS. This suggests that the energy costs associated with $DPA_{\geq 3MET}$ may have a greater impact on the individual cardiometabolic profile, than those associated with the volume of DPA alone.

As mentioned above, Solomon *et al.* have recently reported a linear association between lower IS and poorer cardiorespiratory fitness across the entire range of glucose tolerance [9], but the authors did not find conclusive results on the inverse association of cardiorespiratory fitness with the components of BF in patients with overt T2D. Our study adds further evidence against the association of DPA or EE variables with BF in patients with (newly-diagnosed) T2D. Whether physical activity is able to improve insulin secretion while reducing insulin resistance has been object of investigation for several years. However, while there is supportive evidence from some intervention studies on the efficacy of aerobic exercise training at improving BF in patients with T2D [29-31], recent results from the RAED2 study, a randomized clinical trial conducted by our group, did not reveal significant improvements of BF after random assignment to structured aerobic or resistance training in 40 patients with T2D [32]. However, the disparity in the results obtained by these studies might be explained by the different methods employed to estimate BF (surrogate indexes of BF, simple C-peptide concentrations or mathematical modeling) as well as by the different exercise protocols applied, which all hamper the head-to-head comparison.

Major strengths of our study include: a) the use of state-of-art and totally independent

methods [33-35] to assess BF and insulin sensitivity; b) study patients with newly-diagnosed and drug-naïve T2D, thereby minimizing the effect of a long standing disease and limiting the potentially confounding effects of glucose-lowering medications; c) consecutive recruitment of patients among those attending to the VNDS; as such, the wearable armband system was offered to unselected VNDS participants without any accidental or purposely occurring influence by the enrolling physician.

Our analysis, however, has a number of limitations that should be acknowledged, not ultimately the limited sample size. The SWA was available at our institution for a limited period and it was therefore possible to propose its positioning to a limited number of patients only. This was the major issue that prevented us to enroll, although desirable, a larger number of patients and thus to perform more detailed analyses. Our study, indeed, was descriptive in nature and it was not designed to explore causal relationships or to unravel mechanistic insights on the effects of increasing DPA and accompanying EE. Additionally, the absence of a control group without T2D might affect the generalizability of our findings. It is also noteworthy that, although vigorous-intense physical activity may be relevant in the relationship with clinical parameters, none of the study patients exercised above 6 METs, and it therefore remains untested whether high-intensity exercise may give extra benefits over light-to-moderate physical activity in our patient population. There are also other unmeasurable confounding elements that should be mentioned, including the fact that, although our patients did not receive specific physical activity counselling upon the study enrollment (with the only exception of refraining from heavy exercise in the days preceding the metabolic tests), the application of the SenseWear® Armband *per se* may have affected the DPA features in these subjects. Lastly, since the communication of a T2D diagnosis represents an important milestone in the individual life, it is possible that the increased awareness *per se* may have already induced diet and physical activity habits changes, with potential confounding effects on the interpretation of the study results.

As for the novelty of our results, it is noteworthy that the benefits of moderate and unstructured physical activity on insulin sensitivity have been observed in untrained individuals and that the positive relationship with the metabolic health parameters was already evident since diabetes diagnosis. Indeed, the timing of T2D onset and its diagnosis are not necessarily overlapping, and usually the latter follows the former after several weeks or months, if not years. However, in many circumstances, the time of T2D diagnosis represents the only practicable proxy of T2D onset, and a hitherto unparalleled opportunity to detect the first instances of the disruption between IS and BF during the natural history of T2D. Additionally, it could not be excluded that long-term structured physical activity programs may eventually result in measurable improvements of BF.

In conclusion, although further interventional studies are needed to definitely demonstrate whether stepwise incremental levels of moderate DPA effectively improve the individual metabolic health parameters in these patients, our data strengthen the confidence that simple recommendations to increase the time spent in moderate levels of physical activity may be beneficial and easily applicable by virtually every walking patient since the T2D diagnosis.

SUPPORTING INFORMATION

Includes text with supplemental information regarding methods and results, tables S1-S2 and figures S1-S9.

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AUTHOR CONTRIBUTIONS

M. Dauriz and E. Bacchi designed the study, researched and analyzed data and wrote the manuscript. M.L.B. and L.S. researched and analyzed data. C.N. and M.T. researched data and discussed the manuscript. R.C.B., E. Bonora and P.M. designed the study, edited the manuscript and provided substantial contribution to the overall discussion. M. Dauriz and E. Bacchi are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity and the accuracy of the data analysis.

DUALITY OF INTEREST

The authors have no competing interest to declare.

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Table I – Clinical and metabolic features of the study sample.

Variables	Men	Women	All	*p*-value*
N	24	17	41	
Age, years	63 [50-67]	63 [50-67]	62 [52.5-67]	ns
BMI, $Kg \cdot m^{-2}$	29.8 [26.2-32.7]	32.5 [28.1-35.3]	30.8 [26.5-34.3]	ns
Waist circumference, cm	100.5 [91-112]	99 [95-101]	99.5 [92.5-108]	ns
Fat Mass, %	33.5 [26.5-41.6]	41.3 [38.8-43.6]	39.2 [31.6-43.1]	ns
Fasting insulin,mU/L	76.8 [34.7-108.5]	75.6 [53.4-113.7]	76.8 [45.0-104.4]	ns
Fasting plasma glucose, mmol/L	7.1 [6.1-8.9]	7.1 [6.5-8.5]	7.1 [6.4-8.8]	ns
2hr plasma glucose, mmol/L	13.5 [9.9-16.9]	13.3 [10.1-14.3]	13.3 [10.1-15.9]	ns
Glucose-AUC$_{OGTT}$, mmol/L·min	163 [125-217]	139 [128-184]	155 [128-202]	ns
HbA$_{1c\text{-}DCCT}$, %	6.7 [6.2-7.6]	6.9 [6.5-7.3]	6.7 [6.3-7.3]	ns
HbA$_{1c\text{-}IFCC}$, mmol/mol	49.7 [44.3-59.6]	51.9 [47.5-56.3]	49.7 [45.4-56.3]	ns
Total cholesterol, mmol/L	5.1 [4.2-5.7]	5.2 [4.5-5.9]	5.1 [4.3-5.8]	ns
C-HDL, mmol/L	1.0 [0.9-1.2]	1.1 [1.0-1.3]	0.9 [1.1-1.3]	ns
Triglycerides, mmol/L	1.6 [1.0-2.1]	1.2 [1.1-1.9]	1.4 [1.1-2.0]	ns
SBP, mmHg	132 [130-149]	140 [130-150]	140 [130-150]	ns
DBP, mmHg	82 [80-90]	80 [80-90]	80 [80-90]	ns
Lipid-lowering medications, %	25	23.5	24.3	ns
Anti-hypertensive medications, %	62.5	58.8	60.9	ns
Aspirin, %	20.8	29.4	24.4	ns
Smoking status, % (ever/never)	70.8	52.9	63.4	ns
Insulin sensitivity:				
M clamp, µmol/min/m^2 BSA	505 [221-949]	644 [464-748]	630 [371-878]	ns
HOMA-IR, score	3.8 [1.9-7.5]	3.9 [3.1-5.5]	3.9 [2.3-5.9]	ns
Matsuda Index	3.1 [1.6-7.2]	2.5 [1.9-3.5]	2.7 [1.9-4.6]	ns
Beta-Cell Function:				
Derivative Control ($pmol \cdot m^{-2}$ BSA)·($mmol \cdot L^{-1} \cdot min^{-1})^{-1}$	640.1 [191-1100]	94.3 [0-708]	333.8 [0.3-909]	0.06
Proportional Control ($pmol \cdot min^{-1} \cdot m^{-2}$ BSA)	51.5 [32-101]	54.3 [35-85]	53.9 [33-90]	ns
HOMA-B, %	61.8 [35.7-100.3]	58.0 [37.7-123.7]	58.0 [37.1-101.2]	ns
Insulinogenic Index (mU/mmol)	4.0 [2.6-8.6]	5.8 [2.6-7.6]	4.2 [2.2-7.4]	ns
CIR$_{120'}$ ($mU \cdot L/mmol^2$)	0.6 [0.3-1.9]	0.9 [0.2-1.4]	0.6 [0.2-1.6]	ns
Disposition Index[§]	23.2 [12.4-67.1]	27.1 [13.4-36.6]	24.7 [12.7-44.5]	ns
Physical activity (PA)				
PA ≥3 METs, min/day	81.8 [59-145]	53 [32-86]	70 [38-125]	0.05
3< PA <6 METs, min/day	81.8 [59-145]	53 [32-86]	70 [38-125]	0.05
Footsteps, *N* of steps/day	7,718 [5479-1,1202]	8,728 [5,748-10,927]	7,773 [5,748-10,927]	ns
Energy Expenditure (EE)				
EE$_{TOT}$, Cal/day	2,751 [2,402-3,065]	2,229 [2,065-2,365]	2,398 [2,226-2,801]	<0.001
EE ≥3 METs, Cal/day	406 [282-764]	283 [162-428]	364 [238-617]	<0.05
Resting (lying), hours/day	8.3 [7.2-9.7]	8.3 [7.3-9.4]	8.3 [7.3-9.5]	ns
Resting (sleeping), hours/day	6.7 [5.0-7.6]	6.8 [6.2-8.2]	6.8 [5.2-7.9]	ns

Legend: Data are presented as median [I.Q. range]; BMI, Body Mass Index; $HbA_{1c\text{-}DCCT}$, Diabetes Control and Complication Trial-Aligned Hemoglobin A_{1c}; $HbA_{1c\text{-}IFCC}$, International Federation of Clinical Chemistry-Aligned Hemoglobin A_{1c}; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; HOMA-B, Homeostasis Model Assessment of Beta-cell Function; AUC, area under the curve; BSA, Body Surface Area; $CIR_{120'}$, corrected insulin response at 120' of the OGTT; Matsuda Index: $10{,}000/[(Glucose_{0'} \cdot Insulin_{0'}) \cdot (\text{mean OGTT glucose concentration}) \cdot (\text{mean OGTT insulin concentration})]^{1/2}$; METs, METabolic Equivalents; EE_{TOT}, Total Daily Energy Expenditure. [§]Disposition Index was calculated according to the following formula: $DI=(\Delta I_{0'\text{-}120'}/\Delta G_{0'\text{-}120'})$ x Matsuda Index. *Sex differences were evaluated by unpaired two-sample *t*-test or chi-square test for continuous or categorical variables, respectively. Non-Gaussian variables were natural-log transformed.

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Table II – Correlations (Pearson's *r*) between physical activity measures, body composition and metabolic parameters.

Variables	Footsteps *N* of steps/day			DPA ≥3 METs min/day			EE_{TOT} Kcal/day			EE ≥3 METs Kcal/day		
	Men	**Women**	**All**	**Men**	**Women**	**All**	**Men**	**Women**	**All**	**Males**	**Females**	**All**
BMI, Kg/m^2	-0.31	-0.36	-0.31*	-0.28	-0.4	-0.37*	0.25	0.35	0.08	-0.2	-0.29	-0.29
Waist Circumference, cm	-0.31	-0.62*	-0.42*	-0.39	-0.56*	-0.37*	0.24	0.26	0.27	-0.32	-0.42	-0.28
Fat mass, %	-0.14	-0.32	-0.14	-0.25	-0.33	-0.33*	0.02	0.22	-0.12	-0.22	-0.28	-0.29
$HbA_{1c\text{-}DCCT}$, %	-0.05	0.16	0.04	0.09	-0.04	0.03	0.08	0.02	0.02	0.1	0.06	0.06
Fasting Plasma Glucose, mmol/L	0.18	-0.19	0.06	0.14	-0.01	0.13	0.18	0.23	0.21	0.15	0.08	-015
2-hour Plasma Glucose, mmol/L	-0.08	0.11	-0.01	-0.11	-0.04	-0.05	0.01	0.12	0.09	-0.11	0.07	-0.01
Glucose-AUC_{OGTT}, mmol/L·min	0.08	0.08	0.07	-0.01	0.01	0.05	0.06	0.28	0.18	-0.01	0.14	0.09
C-HDL, mmol/L	0.36	0.3	0.33*	0.53*	0.35	0.33*	0.39	0.15	0.10	0.51*	0.38	0.32*
Triglycerides, mmol/L	-0.49*	-0.42	-0.46*	-0.37	-0.4	-0.32*	-0.24	0.12	-0.07	-0.37	-0.34	-0.29
Disposition Index	0.11	0.09	0.04	0.02	0.35	0.08	-0.11	-0.32	-0.05	-0.01	0.15	0.05
Insulin Sensitivity:												
M clamp, $\mu mol/min/m^2_{BSA}$	0.47*	0.55*	0.45*	0.53*	0.49	0.45*	0.38	-0.06	0.18	0.52*	0.42	0.41*
Beta-cell Function:												
Derivative Control $(pmol \cdot m^{-2}_{BSA}) \cdot (mmol \cdot L^{-1} \cdot min^{-1})^{-1}$	-0.32	0.3	0.05	-0.12	0.18	0.09	-0.19	0.29	0.12	-0.13	0.14	0.08
Proportional Control $(pmol \cdot min^{-1} \cdot m^{-2}_{BSA})$	-0.04	0.15	0.04	-0.14	0.18	-0.08	-0.16	0.19	-0.18	-0.14	0.04	-0.11

*Association is significant at two-tailed $p<0.05$; BMI, Body Mass Index; BSA, Body Surface Area; C-HDL, High Density Lipoprotein Cholesterol; EE, Daily Energy Expenditure; $HbA_{1c\text{-}DCCT}$, Diabetes Control and Complication Trial-Aligned Hemoglobin A1c; M clamp, insulin sensitivity; METs, Metabolic equivalents; DPA, daily physical activity. Non-Gaussian variables were natural-Log transformed.

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Table III – Predictors of insulin sensitivity by generalized linear regression models.

Model 1 (R^2=18%, p=0.11)	***Beta***	***P***	**adjusted-R^2**
Footsteps	0.36	0.03	0.13
Age	0.18	0.26	0.03
Sex	-0.03	0.83	0.001
BMI	-0.05	0.75	0.03
Model 2 (R^2=22%, p=**0.05**)			
DPA ≥3 MET	0.44	**0.01**	0.16
Age	0.18	0.23	0.04
Sex	0.12	0.47	0.01
BMI	-0.04	0.80	0.002
Model 3 (R^2=24%, p=**0.03**)			
EE_{TOT}	0.52	**0.006**	0.19
Age	0.21	0.17	0.05
Sex	0.32	0.09	0.07
BMI	0.29	0.06	0.09
Model 4 (R^2=21%, p=0.08)			
EE ≥3 MET	0.41	0.02	0.15
Age	0.19	0.23	0.04
Sex	0.12	0.45	0.02
BMI	-0.09	0.59	0.01

Multivariable linear regression models (dependent variable: insulin sensitivity; independent variables: age, sex, BMI, footsteps (Model 1), DPA ≥3 MET (Model 2), EE_{TOT} (Model 3), EE ≥3 MET (Model 4)).

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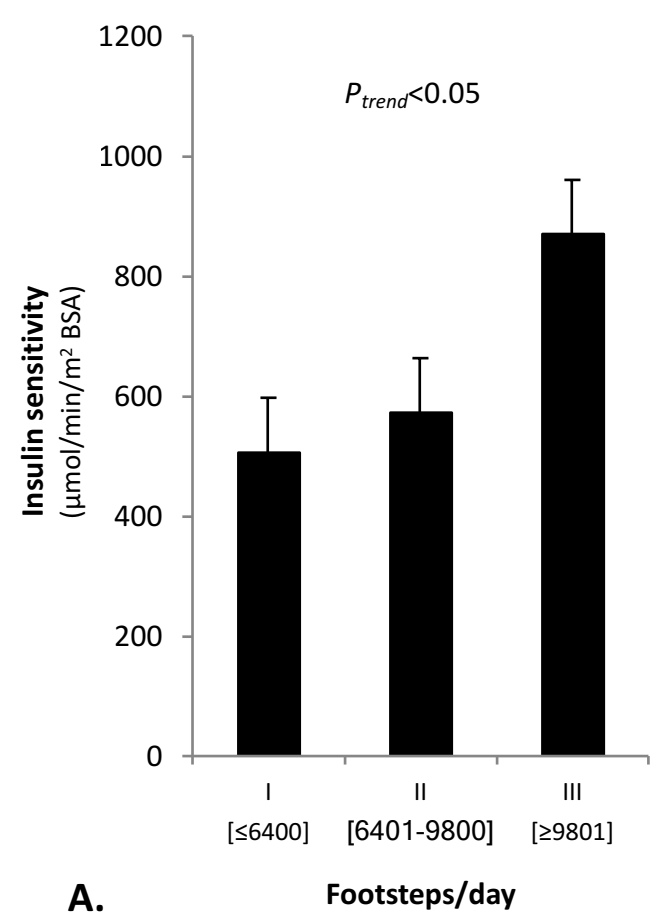
Table IV – Predictors of insulin sensitivity by generalized linear regression models.

Model 1 (R^2=21.3%, p=0.07)	$ß_{std}$	*P*	adjusted-R^2
Footsteps	0.30	0.08	0.15
Age	0.18	0.23	0.03
Sex	-0.07	0.64	0.001
Waist	-0.20	0.23	0.10
Model 2 (R^2=24.2%, p=**0.04**)			
DPA ≥3 MET	0.37	**0.04**	0.16
Age	0.19	0.21	0.03
Sex	0.06	0.71	$1.2*10^{-4}$
Waist	-0.17	0.30	0.10
Model 3 (R^2=35%, p=**0.003**)			
EE_{TOT}	0.56	**0.002**	0.08
Age	0.24	0.09	0.03
Sex	0.20	0.22	$1.2*10^{-4}$
Waist	0.44	**0.003**	0.10
Model 4 (R^2=24%, p=**0.04**)			
EE ≥3 MET	0.35	**0.04**	0.14
Age	0.20	0.20	0.03
Sex	0.05	0.74	$1.2*10^{-4}$
Waist	-0.21	0.19	0.10

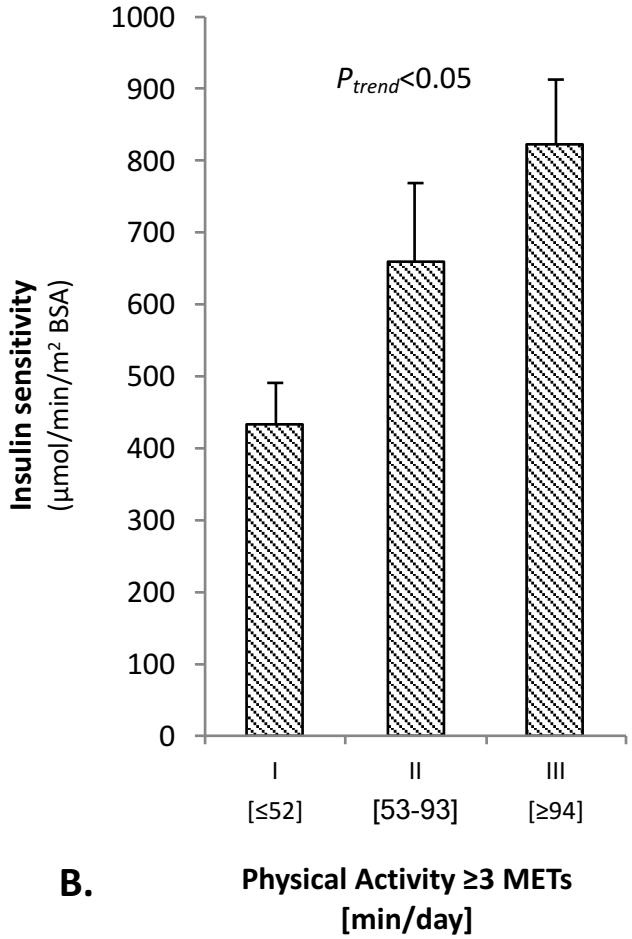
Multivariable linear regression models (dependent variable: insulin sensitivity; independent variables: age, sex, waist, footsteps (Model 1), DPA ≥3 MET (Model 2), EE_{TOT} (Model 3), EE ≥3 MET (Model 4)).

FIGURE LEGEND

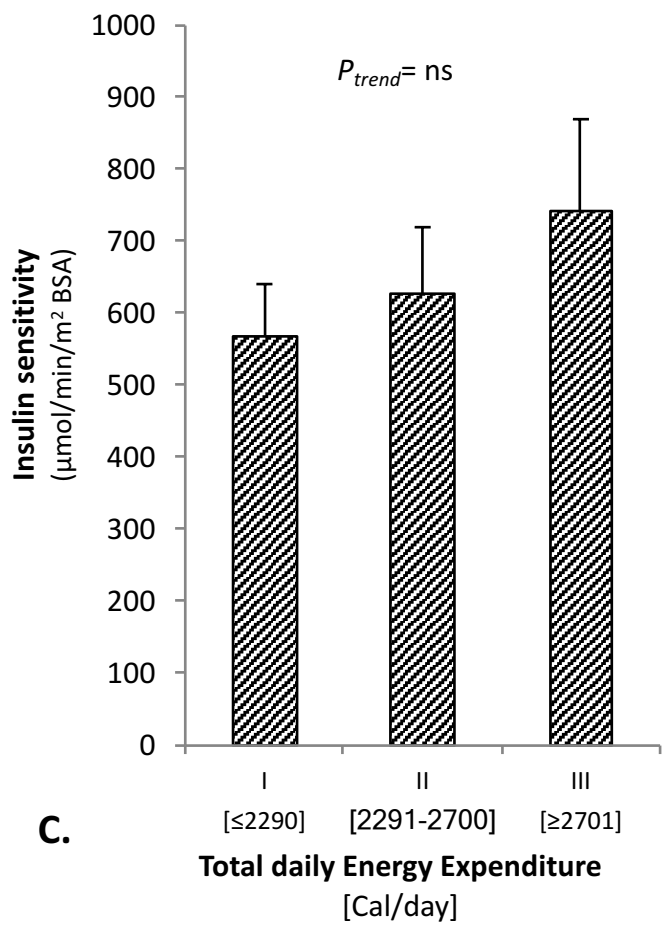
Figure 1 – Distribution of insulin sensitivity across incremental categories of physical activity and energy expenditure measures in patients with newly-diagnosed type 2 diabetes. The figure shows the significant relationship of moderate levels of physical activity and energy expenditure with increasing insulin sensitivity levels, independent of age, sex and adiposity. Categories on the *x*-axes of panels A, B, C and D are expressed, respectively, as tertiles of footsteps, $DPA_{\geq 3MET}$, EE_{TOT} and $EE_{\geq 3MET}$, and calculated from the SWA records obtained in the whole sample. Data expressed as mean±SEM. One-way ANOVA adjusted for age, sex, BMI.


1200
1000
800
600
400
200
0
P_{trend}<0.05
Insulin sensitivity
(µmol/min/m² BSA)
I
[≤6400]
II
[6401-9800]
III
[≥9801]
Footsteps/day

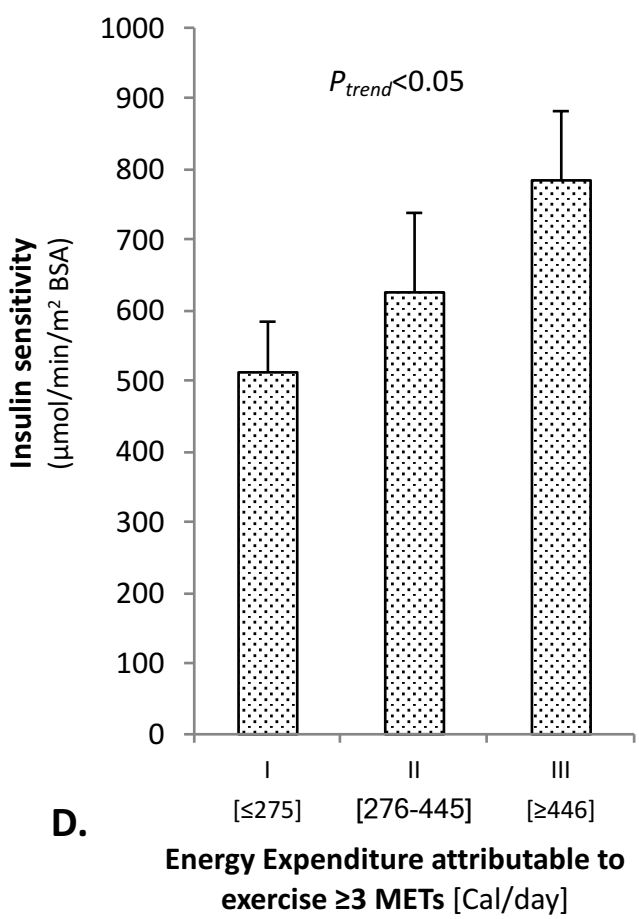
A.


1000
900
800
700
600
500
400
300
200
100
0
P_{trend}<0.05
Insulin sensitivity
(µmol/min/m² BSA)
I
[≤52]
II
[53-93]
III
[≥94]
Physical Activity ≥3 METs
[min/day]

B.


1000
900
800
700
600
500
400
300
200
100
0
P_{trend}= ns
Insulin sensitivity
(µmol/min/m² BSA)
I
[≤2290]
II
[2291-2700]
III
[≥2701]
Total daily Energy Expenditure
[Cal/day]

C.


1000
900
800
700
600
500
400
300
200
100
0
P_{trend}<0.05
Insulin sensitivity
(µmol/min/m² BSA)
I
[≤275]
II
[276-445]
III
[≥446]
Energy Expenditure attributable to
exercise ≥3 METs [Cal/day]

D.

Title: Association of free-living physical activity measures with metabolic phenotypes in type 2 diabetes at the time of diagnosis. The Verona Newly Diagnosed Type 2 Diabetes Study (VNDS) 8.

Authors: Dauriz M*, Bacchi E*, Boselli L, Santi L, Negri C, Trombetta M, Bonadonna RC, Bonora E, Moghetti P.

Highlights:

- Physical activity is a major determinant of glucose homeostasis.
- Whether free-living physical activity impacts insulin sensitivity, beta-cell function or both is poorly understood.
- We tested these questions in deeply phenotyped, newly-diagnosed type 2 diabetes.
- Clamp-assessed insulin sensitivity was linearly associated with moderate physical activity, while model-derived beta-cell function was not.
- These data highlight that moderate and unstructured physical activity shows its metabolic benefits in untrained individuals since diabetes diagnosis.
- Whether structured programs of moderate physical activity may also improve beta-cell function warrants further investigation