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Low-grade inflammation in persons with recently diagnosed type 2 diabetes: The role of abdominal adiposity and putative mediators

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Abstract

Aims: To determine the magnitude of the association between abdominal adiposity and low-grade inflammation in persons with recently diagnosed type 2 diabetes (T2D) and to determine to what extent this association is mediated by low physical activity level, hyperinsulinaemia, hyperglycaemia, dyslipidaemia, hypertension, and comorbidities.

Materials and Methods: We measured waist circumference, clinical characteristics, and inflammatory markers i.e. tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and high-sensitivity C-reactive protein (hsCRP), in >9000 persons with recently diagnosed T2D. We applied multiple mediation analysis using structural equation modelling, with adjustment for age and sex.

Results: Waist circumference as a proxy for abdominal adiposity was positively associated with all inflammatory markers. Hence, a one-standard deviation (SD) increase in waist circumference (SD = 15 cm) was associated with a 22%, 35%, and 46% SD increase in TNF-α (SD = 1.5 pg/mL), IL-6 (SD = 4.4 pg/mL), and hsCRP (SD = 6.9 mg/L), respectively. The level of hyperinsulinaemia assessed by fasting C-peptide

was quantitatively the most important mediator, accounting for 9%–25% of the association between abdominal adiposity and low-grade inflammation, followed by low physical activity (5%–7%) and high triglyceride levels (2%–6%). Although mediation of adiposity-induced inflammation by greater comorbidity and higher glycated haemoglobin levels reached statistical significance, their impact was minor (1%–2%).

Conclusions: In persons with recently diagnosed T2D, there was a clear association between abdominal adiposity and low-grade inflammation. A considerable part (20%–40%) of this association was mediated by other factors, with hyperinsulinaemia as a potentially important driver of adiposity-induced inflammation in T2D.

KEYWORDS

body composition, cohort study, insulin resistance, observational study, population study, type 2 diabetes

1 | INTRODUCTION

In the general population, obesity, and in particular abdominal adiposity, is associated with systemic low-grade inflammation, as demonstrated by increased circulating levels of inflammatory markers such as tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and high-sensitivity C-reactive protein (hsCRP).[1–3] Increased systemic low-grade inflammation is further associated with an increased risk of cardiovascular disease (CVD).[4,5] Abdominal obesity is also associated with inflammatory markers in persons with type 2 diabetes (T2D).[6,7] However, the magnitude of this association and the role of putative mediators that may contribute to this association remains to be established in a large population recently diagnosed with T2D.

Recent studies in animals and human cell lines have shown that hyperinsulinaemia and insulin resistance can promote inflammation in adipose tissue, and hence systemic low-grade inflammation.[8,9] Other studies suggest that a low physical activity level is associated with a higher degree of low-grade inflammation[10,11] and that hypertension, dyslipidaemia, hyperglycaemia, and comorbidities could play a role in the circulating levels of inflammatory markers.[12–15] However, it is unknown if these clinical and metabolic factors, known to be altered in persons with T2D, could mediate the association between abdominal obesity and systemic low-grade inflammation.

In the present study, we applied multiple mediation analysis to determine the association between abdominal adiposity and systemic low-grade inflammation in a large population recently diagnosed with T2D, and to determine to what extent modifiable clinical and metabolic factors altered in T2D contribute to this association.

2 | MATERIALS AND METHODS

2.1 | Study population

This cross-sectional study used data from the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort. Since 2010, study participants have been enrolled from general practice or hospital specialist outpatient clinics in Denmark and enrolment is ongoing.[16] Upon study enrolment, participants undergo a physical examination, including blood sampling and measures of anthropometry, and fill out a questionnaire regarding lifestyle and family history of T2D. In December 2021, plasma samples in the biobank for the entire DD2 cohort were analysed for TNF-α, IL-6, and hsCRP levels ($N > 9000$). Study participants gave written informed consent prior to participation. The study was approved by the Regional Committee on Medical Health Ethics (S-20100082) and the Danish Data Protection Agency (2008-58-0035) and was conducted in concordance with the Helsinki Declaration II.

2.2 | Exposure (abdominal adiposity) and outcomes (inflammatory markers)

We used waist circumference as a proxy for abdominal adiposity as it has been shown to be the best anthropometric predictor of visceral fat compared with body mass index (BMI) and waist-to-hip ratio.[17] Waist circumference was measured at study enrolment with measuring tape between the lower rib margin and the iliac crest. Using the Meso Scale Discovery technique with V-plex validated immunoassays (Meso Scale Diagnostics, Rockville, MD, USA), we measured levels of plasma TNF-α and IL-6 (pg/mL). Intra- and inter-plate coefficients of variation were 5% and 14.4% for TNF-α, and 6.1% and 12.3% for IL-6. We measured serum hsCRP (mg/L) with an enzyme-linked immunosorbent assay technique using an in-house time-resolved immunofluorometric assay, as previously described.[18,19] Intra- and inter-assay coefficients of variation were <5% and <6%, respectively.

2.3 | Mediators

Potential mediators comprised low physical activity level, estimates of hyperinsulinaemia/insulin resistance and glycaemic control, lipids,

blood pressure, and comorbidities. Physical activity was self-reported using the validated Saltin-Grimby Physical Activity Level Scale.[20] Data on glycated haemoglobin (HbA1c), triglycerides, low-density lipoprotein (LDL) cholesterol, and systolic and diastolic blood pressure were obtained from the Danish Diabetes Database of Adults (DDDA) using the hospitals' routine analysis procedures closest to the date of enrolment. Fasting serum C-peptide (pmol/L), as a marker of both hyperinsulinaemia and insulin resistance, was analysed using the Roche C-peptide assay (Roche Diagnostics, Mannheim, Germany). Fasting derived indices of insulin resistance and beta-cell function were estimated using homeostatic model assessment (HOMA2-IR and HOMA2-B, respectively) from fasting C-peptide and plasma glucose levels.[21] Information on comorbidities according to the Charlson comorbidity index, excluding diabetes, was obtained via linkage to the Danish National Patient Registry tracked 10 years back from date of enrolment.[22]

2.4 | Covariates

Covariates comprised diabetes duration, smoking status, alcohol consumption, birth weight, family history of T2D, and genetic predisposition to T2D. Diabetes duration was calculated from the number of days between diabetes diagnosis and study enrolment. Diagnosis of diabetes was obtained from the start-up interview with the general practitioner or was otherwise defined from the first occurring event of the following: (1) first glucose-lowering drug redemption; (2) first diabetes-related contact with the hospital system; (3) first registration in the DDDA; or (4) study enrolment in DD2. Self-reported average weekly alcohol consumption was derived from the start-up interview, whereas self-reported smoking status was obtained from DDDA. Current smoking comprised occasional smoking as well. Alcohol overuse was defined as weekly consumption above 14 units for women and 21 units for men. Birth weight was obtained from midwife journals retrieved from the Danish National Achieves. Family history of T2D in children, siblings, parents, grandparents, and great-grandparents was self-reported. Genotyping was performed using the Global Screening Array-24 v2.0 (Illumina, San Diego, CA, USA). After removing individuals and variants with >5% missingness in quality control, the genotype data were imputed using the Haplotype Reference Consortium reference panel build GRCh37. The polygenic risk score was calculated using a published weighted score,[23] and we were able to include 94% of the variants in the score. Genetic data were only available for a subset of the study population ($n = 3052$). Glutamic acid decarboxylase-autoantibodies (GAD-ab) were measured using the AESKULISA GAD65 kit (AESKU Diagnostics, Wendelsheim, Germany), with high sensitivity, specificity and positive predictive value.[24]

2.5 | Statistical analysis

Due to a predominance of low values, the values of all inflammatory markers were transformed according to the natural logarithm to obtain variance homogeneity and normal distributed residuals in the statistical analyses. To further accommodate the statistical requirements of the mediation analysis and for ease of interpretation, all included variables were standardized according to the standard deviation (SD) scale. Four observations of unrealistic waist circumference (<50 cm) were removed from the dataset after comparing waist circumference with measures of weight, height, and hip circumference. Differences in characteristics of the study population across sex-specific quartiles of abdominal adiposity were assessed using the Kruskal–Wallis test.

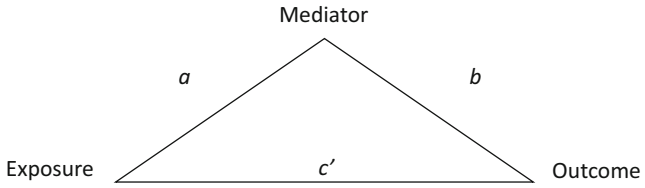

FIGURE 1 Theoretical framework of how mediation is estimated. The total effect (c) is the sum of the direct path (c') and indirect path(s) (path a* path b).

Initially, we applied multiple linear regression analyses, adjusted for age and sex, to determine the associations between degree of abdominal adiposity and low-grade inflammation. This association reflects the total effect and can be denoted as *path c*. Likewise, we regressed abdominal adiposity on the potential mediators of low-grade inflammation, denoted as the first mediating path (*path a*), and we regressed the potential mediators on low-grade inflammation, denoted as the second mediating path (*path b*). The potential mediator was included in a subsequent mediation model only if both *path a* and *path b* were statistically significant. Together, *path a* and *path b* constitute the indirect effect of the association between, in this case, abdominal adiposity and low-grade inflammation, denoted as the direct effect (*path c'*). Hence, the total effect is the sum of the direct and indirect paths (Figure 1). The mediators and confounders were identified based on a directed acyclic graph (Supplementary Appendix S1).

Subsequently, we carried out both single and multiple mediation analyses using structural equation modelling with post hoc estimations of the total, direct as well as indirect effect as proposed by Zhao et al.[25] This two-step approach includes (1) determining *path a*, *path b* and *path c'* and (2) estimating the total effect (*path c*) by performing bootstrapping with 1000 replications to obtain more precise confidence intervals of the indirect and total effects. First, we performed single mediation models, adjusted for age and sex, for each potential mediator including physical activity, C-peptide, HOMA2-B andHOMA2-IR, HbA1c, LDL cholesterol, triglycerides, diastolic and systolic blood pressure, and comorbidities. Due to the varying number of observations, the consequence of many data sources and general data incompleteness, the direct effects differed across mediators. Therefore, we performed multiple mediation analysis including only significant mediators from the single mediation analyses to study the concepts under the same conditions. We checked for multicollinearity

TABLE 1 Characteristics of the study population stratified by sex-specific quartiles of abdominal adiposity.

Quartiles of waist circumference	Q1 ♀ 57–94 cm ♂ 53–100 cm	Q2 ♀ 101–109 cm ♂ 95–103 cm	Q3 ♀ 110–119 cm ♂ 104–114 cm	Q4 ♀ 120–209 cm ♂ 115–197 cm	*p* value
N (%)	2557 (26.5)	2382 (24.7)	2379 (24.7)	2322 (24.1)	
Males, *n* (%)	1490 (58.3)	1431 (60.1)	1383 (58.1)	1349 (58.1)	0.44
Age, years	63.2 (54.3; 69.7)	62.8 (54.0; 69.8)	61.6 (53.0; 68.4)	58.1 (49.8; 65.8)	<0.01
IL-6, pg/mL	0.9 (0.7; 1.5)	1.1 (0.8; 1.7)	1.3 (0.9; 1.9)	1.6 (1.1; 2.3)	<0.01
TNF-α, pg/mL	0.9 (0.7; 1.1)	1.0 (0.8; 1.2)	1.0 (0.8; 1.2)	1.1 (0.9; 1.3)	<0.01
Serum hsCRP, mg/L	1.0 (0.5; 2.5)	1.7 (0.8; 3.5)	2.3 (1.1; 4.7)	3.8 (1.8; 7.5)	<0.01
Diabetes duration, years	0.9 (0.2; 2.5)	0.9 (0.1; 2.7)	0.8 (0.1; 2.5)	0.7 (0.1; 2.6)	0.01
Weight, kg	75 (67; 84)	88 (80; 95)	97 (89; 106)	115 (105; 128)	<0.01
Height, cm	171 (164; 178)	173.0 (165; 179)	173 (166; 180)	175 (168; 181)	<0.01
BMI, kg/m^2	25.7 (23.8; 27.6)	29.3 (27.5; 31.2)	32.3 (30.2; 34.5)	37.8 (34.9; 41.5)	<0.01
Waist circumference, cm	92 (87; 96)	103 (100; 106)	112 (110; 115)	125 (121; 132)	<0.01
Hip circumference, cm	98 (94; 102)	105 (102.0; 110)	111 (107; 116)	122 (116; 131)	<0.01
Birth weight, g	3300 (3000; 3600)	3400 (3000; 3700)	3400 (3050; 3700)	3500 (3100; 3780)	<0.01
Family history of T2D, *n* (%)	1379 (53.9)	1264 (53.1)	1295 (54.4)	1209 (52.1)	0.41
Polygenic risk score for T2D	52.7 (52.6; 52.8)	52.7 (52.6; 52.8)	52.7 (52.6; 52.8)	52.7 (52.6; 52.8)	0.06
Smoking status, *n* (%)					
Never	768 (49.1)	644 (46.4)	637 (46.1)	532 (43.1)	
Former	482 (30.8)	502 (36.2)	481 (34.8)	453 (36.7)	
Current	313 (20.0)	242 (17.4)	264 (19.1)	248 (20.1)	0.01
Alcohol overuse, *n* (%)	133 (5.2)	147 (6.2)	159 (6.7)	133 (5.7)	0.15
Leisure-time physical activity, *n* (%)					
Sedentary	246 (9.6)	332 (14.0)	477 (20.1)	660 (28.5)	
Light activity	1609 (63.1)	1520 (64.0)	1506 (63.5)	1389 (59.9)	
Moderate/vigorous activity	696 (27.3)	522 (22.0)	390 (16.4)	270 (11.6)	<0.01
Systolic BP, mmHg	130 (120; 139)	130 (124; 140)	131 (125; 141)	130 (124; 140)	<0.01
Diastolic BP, mmHg	80 (71; 84)	80 (75; 85)	80 (75; 87)	80 (75; 88)	<0.01
HbA1c, mmol/mol	48.6 (43.2; 54.1)	48.6 (44.3; 55.2)	49.7 (44.3; 56.3)	50.8 (45.4; 58.5)	<0.01
HbA1c, %	6.6 (6.1; 7.1)	6.6 (6.2; 7.2)	6.7 (6.2; 7.3)	6.8 (6.3; 7.5)	<0.01
Glucose, mmol/L	6.9 (6.2; 7.9)	7.1 (6.3; 8.2)	7.3 (6.5; 8.3)	7.3 (6.6; 8.5)	<0.01
C-peptide, pmol/L	858 (654; 1151)	1089 (854; 1427)	1269 (1015; 1665)	1531 (1219; 1937)	<0.01
HOMA2-B, %	80.2 (61.1; 103.5)	90.9 (69.8; 116.9)	98.9 (76.0; 127.2)	108.8 (83.4; 141.5)	<0.01
HOMA2-IR	2.1 (1.6; 2.8)	2.7 (2.1; 3.5)	3.1 (2.5; 4.1)	3.8 (3.0; 4.9)	<0.01
LDL cholesterol, mmol/L	2.2 (1.7; 2.8)	2.2 (1.7; 2.8)	2.2 (1.7; 2.8)	2.2 (1.7; 2.8)	0.90
Triglycerides, mmol/L	1.3 (1.0; 1.9)	1.6 (1.2; 2.3)	1.8 (1.3; 2.5)	1.9 (1.4; 2.7)	<0.01
Comorbidities, *n* (%)					
No	1590 (71.9)	1389 (69.8)	1270 (65.8)	1215 (66.9)	
1	316 (14.3)	306 (15.4)	340 (17.6)	336 (18.5)	
2 or more	304 (13.8)	295 (14.8)	320 (16.6)	265 (14.6)	<0.01
Pre-existing CVD, *n* (%)	388 (17.6)	373 (18.7)	396 (20.5)	322 (17.7)	0.07
Any glucose-lowering drugs, *n* (%)	1564 (70.8)	1470 (73.9)	1469 (76.1)	1421 (78.2)	<0.01
Metformin, *n* (%)	1501 (67.9)	1502 (67.9)	1503 (67.9)	1504 (67.9)	<0.01
Sulphonylureas, *n* (%)	94 (4.3)	75 (3.8)	86 (4.5)	85 (4.7)	0.55

(Continues)

TABLE 1 (Continued)

Quartiles of waist circumference	Q1 ♀ 57–94 cm ♂ 53–100 cm	Q2 ♀ 101–109 cm ♂ 95–103 cm	Q3 ♀ 110–119 cm ♂ 104–114 cm	Q4 ♀ 120–209 cm ♂ 115–197 cm	*p* value
GLP-1 analogues, *n* (%)	46 (2.1)	62 (3.1)	114 (5.9)	138 (7.6)	<0.01
SGLT2 inhibitors, *n* (%)	17 (0.8)	28 (1.4)	28 (1.5)	30 (1.7)	0.07
Insulin, *n* (%)	96 (4.3)	103 (5.2)	103 (5.3)	137 (7.5)	<0.01
Any CVD drug, *n* (%)	1616 (73.1)	1521 (76.4)	1561 (80.9)	1447 (79.7)	<0.01
Antihypertensive drugs, *n* (%)	888 (40.2)	926 (46.5)	1026 (53.2)	991 (54.6)	<0.01
Statins, *n* (%)	1140 (51.6)	1021 (51.3)	1034 (53.6)	952 (52.4)	0.48

Abbreviations: BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; GLP-1, glucagon-like peptide-1; HbA1c, glycated haemoglobin; HOMA2-B, homeostatic model assessment of beta-cell function; HOMA2-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; SGLT2, sodium-glucose cotransporter-2; T2D, type 2 diabetes; TNF-α, tumour necrosis factor-alpha.

among all mediators as high correlation would violate the statistical model assumption of the multiple mediation model. If mediators were highly correlated, we excluded the least significant mediator. The direct and indirect effects were presented in both absolute and relative terms. In absolute terms, the beta-coefficients resembled 'X' SD change in levels of inflammatory markers on the logarithmic scale by a 1-SD increase in waist circumference. In relative terms, we presented both the ratio of the indirect to the total effect (RIT) as well as the direct effect to the total effect.

To account for more extensive confounder adjustment, diabetes duration, smoking, alcohol, birth weight, and family history of T2D were included in addition to age and sex (Model 2), and ultimately a polygenic risk score for T2D was included as well (Model 3). To minimize biased results due to selection of study participants with extended confounder adjustment, post hoc sensitivity analyses were performed by restricting the main analysis to the reduced sample sizes of Models 2 and 3. Two additional sensitive analyses were further conducted to investigate if the exclusion of persons with suspected acute inflammation (hsCRP >10 mg/L) or autoimmune diabetes (GAD-ab >20 IU/mL) would change the estimates of the total, direct, and indirect effects.

Data were analysed using Stata/BE 17.0 (StataCorp, College Station, TX, USA) with a two-sided *p* value of 0.05. For the mediation analysis the Stata package medsem was applied.

3 | RESULTS

3.1 | Descriptive statistics

Younger age and higher levels of inflammatory markers, anthropometric measures, sedentary lifestyle, hyperinsulinaemia, insulin resistance, beta-cell function, hyperglycaemia, and triglycerides, as well as more frequent use of any glucose-lowering, CVD or antihypertensive drugs, were associated with higher levels of sex-adjusted waist circumference (Table 1). We found that waist circumference was more strongly associated with inflammatory markers compared with BMI and waist-to-hip ratio (data not shown).

3.2 | Single mediation model

Regression analyses, adjusted for age and sex, revealed significant associations between waist circumference and inflammatory markers. Hence, a 1-SD increase in waist circumference (SD = 15 cm) was associated with a 22%, 35%, and 46% SD increase in TNF-α (SD = 1.5 pg/mL), IL-6 (SD = 4.4 pg/mL), and hsCRP (SD = 6.9 mg/L), respectively. Of all the tested paths between exposure → mediator (*path a*) and mediator → outcome (*path b*) only LDL cholesterol among the chosen potential mediators (see above) was not significantly associated with either waist circumference or TNF-α and IL-6 (data not shown). The remaining potential mediators comprising fasting C-peptide, HOMA2-B, HOMA2-IR, physical activity, triglycerides, HbA1c, diastolic and systolic blood pressure, and comorbidities were included in a single mediation analysis performed for each mediator separately (Supplementary Appendix S2–S4). These results showed significant mediation by all the abovementioned factors except diastolic and systolic blood pressure. However, due to multicollinearity between fasting C-peptide, HOMA2-B and HOMA2-IR, only fasting C-peptide was included as a marker of hyperinsulinaemia in the multiple mediation model. Fasting C-peptide was selected over HOMA2-B and HOMA2-IR because of its higher indirect effect (RIT = 12.3%–35.3% vs. RIT = 2.4%–15.8% for HOMA2-B, and RIT = 12.1%–28.1% for HOMA2-IR).

3.3 | Multiple mediation model

In multiple mediation models, including fasting C-peptide, physical activity, triglycerides, HbA1c, and comorbidities (Figures 2–4), fasting C-peptide was the strongest mediator, explaining 9%–25% of the association between waist circumference and low-grade inflammation, followed by physical activity (5%–7%), triglycerides (1%–6%), HbA1c (1%), and comorbidities (1%–2%).

In total, when adding up the RIT of the indirect effects, 39.8%, 25.3%, and 19.7% of the association between waist circumference and circulating levels of TNF-α, IL-6, and hsCRP were explained by the studied mediators, respectively (Figures 2–4). Serum hsCRP

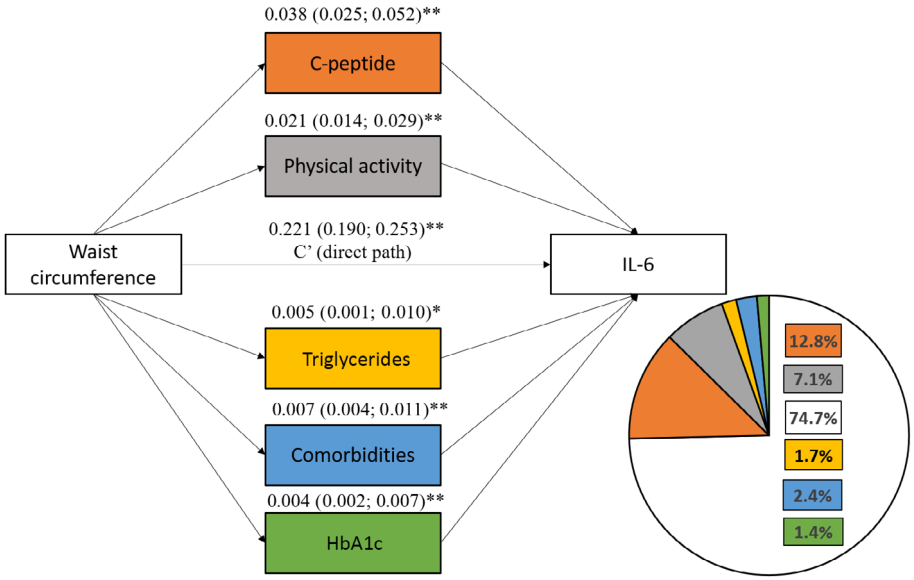

FIGURE 2 Estimates of multiple mediation of the association between waist circumference and interleukin-6 (IL-6), adjusted for age and sex. $N = 4500$. Beta-coefficients resemble 'X' standard deviation (SD) change in levels of inflammatory marker on the logarithmic scale by a 1-SD increase in waist circumference. Percentages in the pie chart represent the ratio of the indirect to the total effect, as well as the direct effect to the total effect. *$p = 0.03$. **p <0.01. HbA1c, glycated haemoglobin.

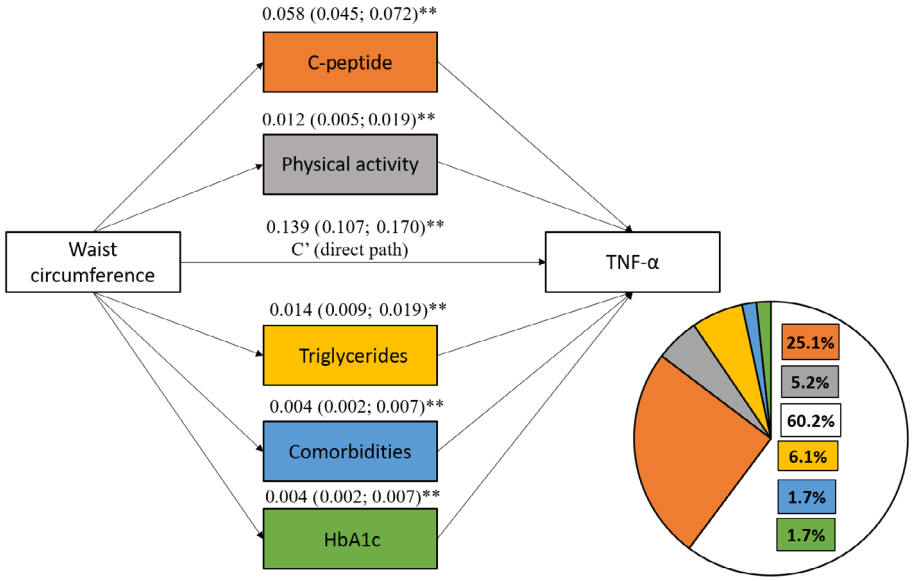

FIGURE 3 Estimates of multiple mediation of the association between waist circumference and tumour necrosis factor-alpha (TNF-α) adjusted for age and sex. $N = 4505$. Beta-coefficients resemble 'X' standard deviation (SD) change in levels of inflammatory marker on the logarithmic scale by a 1-SD increase in waist circumference. Percentages in the pie chart represent the ratio of the indirect to the total effect, as well as the direct effect to the total effect. *p <0.01. HbA1c, glycated haemoglobin.

showed the strongest total (β = 0.390) and direct effects (β = 0.313), whereas plasma TNF-α showed the weakest total (β = 0.231) and direct effects (β = 0.139) but a strong degree of mediation in return. However, when examining estimates from the multiple mediation models, we discovered almost identical absolute indirect effects for IL-6 and hsCRP. The pattern of mediation between waist circumference and plasma TNF-α was different, although the minor indirect effect of HbA1c was similar across all inflammatory markers.

To examine the influence of diabetes duration, smoking, alcohol, birth weight, family history of T2D, and genetic predisposition to T2D, we performed the multiple mediation analyses with extended confounder adjustment to observe if the total, direct and indirect effects changed under these conditions. Hence, in Models 2 and 3, we obtained overall comparable estimates of total, direct and indirect effects to the parsimonious model, adjusted for age and sex. Triglycerides did not mediate the association between waist circumference and IL-6 in Model 2, resulting in absolute and relative higher mediation by the remaining mediators (Supplementary Appendix S5). Furthermore, estimates of total, direct and indirect effects tended to increase by extended confounder adjustment for IL-6 but decrease for TNF-α, whereas total and direct effects increased, and indirect effects decreased for hsCRP in Model 3 (Supplementary Appendix S6). However, fasting C-peptide (8%–25%) and physical activity level (5%–9%) remained the most important mediators, followed by triglycerides (1%–6%), HbA1c (1%–2%) and comorbidities (1%–2%), despite adjusting for potential confounding.

3.4 | Sensitivity analyses

Restricting the study sample to the sample sizes derived from Model 2 (Supplementary Appendix S7) and Model 3 (Supplementary

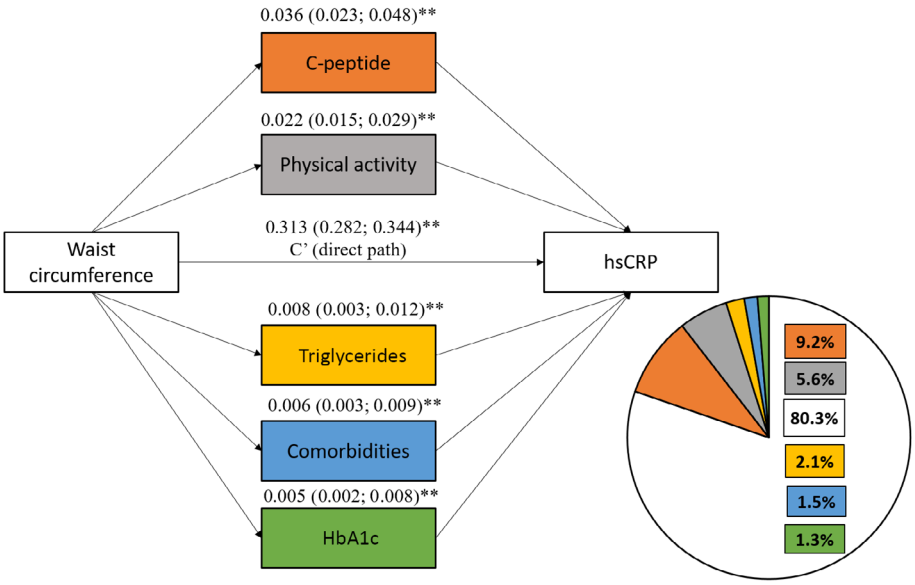

FIGURE 4 Estimates of multiple mediation of the association between waist circumference and high-sensivity C-reactive protein (hsCRP), adjusted for age and sex. $N = 4505$. Beta-coefficients resemble 'X' standard deviation (SD) change in levels of inflammatory marker on the logarithmic scale by a 1-SD increase in waist circumference. Percentages in the pie chart represent the ratio of the indirect to the total effect, as well as the direct effect to the total effect. *p <0.01. HbA1c, glycated haemoglobin.

Appendix S8), we obtained comparable estimates of total, direct and indirect effects to the estimates derived from the parsimonious model. We observed slightly higher total effects with reduced sample sizes for plasma IL-6 and serum hsCRP, whereas total effects were reduced for plasma TNF-α. Plasma triglyceride level was no longer a significant mediator of the association between waist circumference and plasma IL-6 when the sample size was reduced. Likewise, HbA1c was no longer a significant mediator of the association between waist circumference and plasma TNF-α when the sample size was reduced equal to Model 3 (Supplementary Appendix S8).

When we excluded persons with indication of acute inflammation (hsCRP ≥10 pg/mL), we found the same structural pattern of associations, although the total, direct and indirect effects were slightly attenuated (Supplementary Appendix S9). The association between waist circumference and plasma TNF-α was less impacted by this exclusion. Excluding persons with indication of autoimmune diabetes (GAD-ab >20 IU/mL) did not change the structural pattern of associations or attenuate the effects substantially either (Supplementary Appendix S10).

4 | DISCUSSION

The aim of the present study was to decompose the association between abdominal adiposity and low-grade inflammation into direct and indirect effects in persons with recently diagnosed T2D. Apart from a direct effect of abdominal adiposity on low-grade inflammation, we hypothesized that this association could in part be mediated by clinical and metabolic factors related to the T2D diagnosis. Consistent with our hypothesis, we found that abdominal adiposity was positively associated with circulating levels of both TNF-α, IL-6, and hsCRP in a large population with recently diagnosed T2D. Moreover, the results from our multiple mediation analyses demonstrated that clinical and metabolic factors, in particular fasting hyperinsulinaemia and low physical activity level, mediated the association between abdominal adiposity and systemic low-grade inflammation in persons with T2D. Indeed, we found these factors explained 20%–40% of the total association between abdominal adiposity and systemic low-grade inflammation. These results suggest that targeting not only abdominal adiposity but also hyperinsulinaemia/insulin resistance and physical inactivity could reduce systemic low-grade inflammation in persons recently diagnosed with T2D. At present, the most promising interventions to reduce hyperinsulinaemia and insulin resistance include weight loss induced either by diets, bariatric surgery or incretin-based therapies as well as increased physical activity. In addition, further studies are needed to establish if these mediations between abdominal adiposity and low-grade inflammation play a role in the increased risk of CVD observed in persons with T2D.

A mechanistic study has reported that obesity-induced insulin resistance in mice precedes macrophage accumulation and inflammation in adipose tissue.[9] This result was further supported by the observed correlation between HOMA2-IR and activation of pro-inflammatory macrophages in visceral adipose tissue from individuals with a BMI above 30 kg/m^2.[9] Our findings lend support to this hypothesis, although we were unable to isolate the role of hyperinsulinaemia from the role of insulin resistance. Interestingly, human white adipose tissue was recently shown to display selective insulin resistance in the obese state.[26] Thus, it is possible that hyperinsulinaemia and insulin resistance are mutually interrelated by both promoting systemic low-grade inflammation.[27] In line with this, evidence for a role of hyperinsulinaemia was provided in a study showing that a 50% pharmacological reduction of circulating insulin by diazoxide or streptozotocin in obese mice resulted in decreased adipose tissue inflammation.[8] In the present study, we provide evidence for a potential additive effect of hyperinsulinaemia in adiposity-induced low-grade inflammation in a large cohort of patients with recently diagnosed T2D, and, hence in a population with a higher degree of insulin resistance than in non-diabetic obese populations. To the best of our

knowledge, the contribution of fasting hyperinsulinaemia to the association between abdominal adiposity and low-grade inflammation has not previously been reported in a T2D population. Since our findings provide evidence that increased fasting hyperinsulinaemia in T2D mediates the association between abdominal adiposity and systemic low-grade inflammation, this mechanism might also contribute to the residual CVD risk in T2D.[28]

Both increased abdominal adiposity and decreased physical activity are associated with systemic low-grade inflammation.[29] Although physical inactivity contributes to chronic inflammation induced by visceral fat accumulation, physical inactivity has also been shown to increase circulating levels of IL-6 and CRP independent of obesity status.[30] Here, we evaluated a potential role of physical inactivity in mediating the association between abdominal adiposity and low-grade inflammation. We found that low physical activity level accounted for 5%–7% of the association between abdominal adiposity and circulating markers of low-grade inflammation in a large population with recently diagnosed T2D. Although regular physical activity may counterbalance obesity-induced inflammation by direct effects on either abdominal obesity or low-grade inflammation, our results provide evidence that the impact of physical activity on the association between abdominal adiposity and low-grade inflammation is relatively small in our T2D population. Whether this is due to the fact that physical activity was self-reported remains to be established in future studies using a more rigorous estimation of the physical activity level, for example, by accelerometers. A meta-analysis of randomized controlled exercise trials in T2D patients reported significant reductions in circulating levels of IL-6, CRP and TNF-α, with the largest reduction observed in TNF-α.[31] One of the studies included in the meta-analysis reported an exercise-induced reduction in hsCRP independent of the changes in BMI or waist circumference.[32] However, it is unclear to what extent weight loss or reduction in abdominal adiposity contributed to the positive effect of physical activity on these inflammatory markers. Thus, further prospective studies are necessary to establish a role of physical activity in the association between abdominal adiposity and markers of systemic low-grade inflammation in T2D.

As plasma triglyceride levels correlate with the degree of hyperinsulinaemia due to selective insulin resistance in the liver,[33] and hyperinsulinaemia may promote inflammation in adipose tissue,[8] it is reasonable to suggest a role of triglycerides in the accumulation of systemic low-grade inflammation. High levels of triglycerides are a metabolic derangement frequently observed in persons with T2D, and this is further aggravated in the presence of abdominal obesity.[34] We found that triglyceride levels accounted for 1%–6% of the total effect of abdominal adiposity on markers of low-grade inflammation. A previous study has reported an association between higher LDL-triglycerides and higher levels of IL-6 and hsCRP.[35] However, the association between plasma triglycerides and systemic low-grade inflammation in T2D patients is only sparsely examined, and further studies are therefore needed to confirm a role of triglycerides in the association between abdominal adiposity and low-grade inflammation.

A strength of this study is the large study population consisting of a representative sample of persons with recently diagnosed T2D enrolled from the primary healthcare.[16] We believe these results can be generalized to other populations with recent-onset T2D, although, other non-European populations may be more or less predisposed to abdominal obesity, which may change the strength of the direct and indirect effects. Another strength is the use of national health registry data from the Danish Health Data Authority, providing us with complete and highly accurate data on hospital diagnoses and prescriptive medicine use. We further applied waist circumference, which is a better proxy of abdominal obesity than BMI or waist-to-hip ratio.[17]

Our study focused on the association between abdominal adiposity and low-grade inflammation and the potential contribution of selected clinical and metabolic factors related to T2D to this association. This means that any direct effects of these factors on the degree of abdominal adiposity or low-grade inflammation were not evaluated. This study was further limited by its cross-sectional study design. The assumption of mediation requires temporality, and our inference of causality is hampered by all data being measured at study enrolment. However, we constructed a directed acyclic graph to illustrate and justify how we operationalized our theories. Another limitation related to the mediation analysis is the simulated data, as the mediation model relies on counterfactuals. Consequently, we performed a large number of Monte Carlo replications to ensure our estimates had more precise confidence intervals. The use of fasting C-peptide is another potential limitation in the interpretation of our data because this measure of hyperinsulinaemia cannot clearly be separated from insulin resistance. A surrogate measure of insulin resistance, HOMA2-IR, also showed mediating effects, although to a weaker extent. Furthermore, the use of self-reported physical activity rather than, for example, accelerometer-based assessment of physical activity, is a limitation. Finally, we acknowledge that residual confounding due to imperfectly measured, unmeasured, or unknown confounders associated with both abdominal adiposity and inflammation could have influenced some of the observed associations. For example, some misclassification of self-reported smoking and alcohol use may have occurred, but there were no systematic differences in the distribution of smoking and alcohol consumption across quartiles of waist circumference (Table 1), and our main results were unchanged when we adjusted for these factors (i.e., in Models 2 and 3).

In conclusion, our study shows that a higher degree of abdominal adiposity is associated with higher circulating levels of TNF-α, IL-6, and hsCRP in a large population with recently diagnosed T2D. Importantly, we demonstrated that fasting C-peptide, physical activity, triglycerides, comorbidities, and HbA1c partially mediate this association and that these mediating paths are robust to confounder adjustment including lifestyle factors, birth weight, family history of T2D, and genetic predisposition to T2D. Fasting C-peptide was quantitatively the most important mediating factor, suggesting an exacerbating effect of hyperinsulinaemia in adiposity-induced low-grade inflammation. However, the observed associations need to be confirmed in prospective cohort studies or clinical trials enabling causal interpretation of the direct and indirect effects. Thus, insulin-sensitizing interventions including increased physical activity and/or weight loss in larger cohorts with T2D could provide valuable insight into the

complex metabolic interactions between abdominal adiposity and low-grade inflammation. In addition, future prospective studies are needed to examine the associations of clustering of central adiposity, chronic low-grade inflammation, and hyperinsulinaemia/insulin resistance with risk of CVD in persons with recently diagnosed T2D.

AUTHOR CONTRIBUTIONS

Sidsel Domazet performed the statistical analysis, prepared the first draft of the manuscript, and revised the draft. All authors contributed to the interpretation of data and critically revised the content of the draft. Kurt Højlund, Michael Olsen, Allan Vaag and Reimar Thomsen supervised the study. All authors read and approved the manuscript, and gave final approval of the version to be published. Jens Nielsen was the principal manager of the Danish Centre for Strategic Research in Type 2 Diabetes (DD2).

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CONFLICT OF INTEREST STATEMENT

Charlotte Brøns owns stock in Novo Nordisk. Michael Olsen has received payment or honoraria for lectures, presentations or educational events from AstraZeneca, Teva A/S, Novo Nordisk A/S and Boehringer Ingelheim, and has unpaid positions as chairperson of the Danish Hypertension Society and is a Nucleus Member of the Working Group for Prevention and Rehabilitation, Danish Society of Cardiology. Peter Vestergaard is head of research at Steno Diabetes Centre North Denmark funded by the Novo Nordisk Foundation. The remaining authors declare no conflicts of interest relevant to this work.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Danish data protection legislation does not allow sharing of the individual-level personal data used for this study. However, requests to use the primary collected DD2 data can be made at https://dd2.dk/forskning/ansoeg-om-data. Requests to access the Danish health registries used in this study can be sent from researchers at authorized research institutions to the Danish Health Data Authority by e-mail to forskerservice@sundhedsdata.dk.

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