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The effect of metformin on biomarkers associated with breast cancer outcomes: a systematic review, meta-analysis, and dose–response of randomized clinical trials

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Abstract

Purpose Breast cancer is a leading cause of cancer mortality in developed countries. We performed a meta-analysis of randomized clinical trials to investigate the e ect of metformin on biomarkers associated with breast cancer outcomes and to explore the dose–response relationship.

Methods A systematic search was performed from onset of the database to January 2019 in MEDLINE/PubMed, SCOPUS, and Cochrane library to identify randomized clinical trials investigating the impact of metformin on insulin, glucose, CRP, leptin, body mass indices (BMI), cholesterol, Ki-67, and Homeostatic Model Assessment for Insulin-Resistance (HOMA-IR). E ect sizes were expressed as weighted mean di erence (WMD) and 95% confidence intervals (CI) using a random-e ects models. **Results** Nine studies providing 1,363 participants were included in the meta-analysis. Pooled results showed a significant reduction in insulin (WMD: − 0.99 U/ml, 95% CI − 1.66, − 0.33), glucose (WMD: − 1.78 ml/dl, 95% CI − 2.96, − 0.60), CRP (WMD: − 0.60 mg/l, 95% CI − 0.88, − 0.33), HOMA-IR (WMD: − 0.45, 95% CI − 0.77, − 0.11), leptin (WMD: − 2.44 ng/ml, 95% CI − 3.28, − 1.61), BMI (WMD: − 0.55 kg/m^2, 95% CI − 1.00, − 0.11), and Ki-67 (WMD: − 4.06, 95% CI − 7.59, − 0.54). Results of the subgroup analyses showed that insulin, glucose, and BMI decreased more significantly when the duration of administering metformin intervention was above 4 weeks. We did not observe non-linear changes in the dose–response relationship between metformin and biomarkers as outcomes.

Conclusions Breast cancer patients receiving metformin as treatment for diabetes showed significant reduction in levels of insulin, fasting glucose, CRP, HOMA, leptin, BMI, and Ki-67.

Keywords Metformin · Breast cancer · Ki-67 · Insulin · Glucose · BMI

Introduction

Globally, breast cancers are the second most common occur-ring cancers (1.7 million cases, 11.9% of all cancers) next to lung cancer and ranks as the most common cancers among women [1]. According to cancer research UK, by 2030, the global incidence of cancers has been estimated at 23.6 mil-lion new cases [2, 3]. It is the primary cause of mortality in developed countries and the second in developing countries, where the majority of the cases are diagnosed at later stages [4–7].

Cancers affect the morphology of cells resulting in abnormal division which metastasize to form a tumor [5]. Breast tissue consists of fatty, connective, and lymphatic tissues which assist mammary glands in the production of milk. Typically, in the initial stages, benign breast can-cers are asymptomatic with small size tumor size that are easy to treat by surgical excision, supporting screening for early detection [8]. Biomarkers such as p53 gene, HER2, BRCA1 & BRCA2, Ki-67, MUC1, and cyclin D-1 are use-ful for screening, di erentiating tumors, and predicting the response to the therapy [9]. From 2005 to 2014, the inci-dence of breast cancer increased by 1.7%, 0.4%, and 0.3% per year among Asian/Pacific Islander, non-Hispanic black, and Hispanic women, respectively. Thus, novel strategies needed to improve breast cancer outcomes and survival rates [10–12].

Furthermore, outcomes are poorer among patients with type-2 diabetes, which has been associated with a higher cancer risk [13, 14]. There is evidence to suggest that certain diabetic medications such as metformin can modify can-cer risk reducing breast cancer recurrence and mortality, whereas medications such as insulin and its secretagogues are associated with increased recurrence and mortality [15]. A number of observational studies have reported beneficial therapeutic e ects of metformin in preventing breast can-cer reoccurrence and as well as other cancers such as endo-metrial, lung, liver, gastric, and medullary thyroid cancer [16 –21]. The pathways for the e ects of metformin include the reduction of hepatic glucose production which increases hepatic fatty acid oxidation, reducing inflammation, and improving peripheral insulin sensitivity [22– 24]. Collec-tively, this process seems to reduce circulating glucose and insulin levels. However, evidence to support the mechanism of action of metformin is yet to be confirmed [25, 26]. This systematic review and dose–response meta-analysis attempts to summarize the results from existing evidence on the e ects of metformin on biomarkers associated with breast cancer outcomes such as on insulin, glucose, CRP, leptin, body mass indices (BMI), cholesterol, Ki-67, and homeo-static model assessment for insulin-resistance (HOMA-IR).

Methods

Study design and search strategy

This systematic review was performed according to the preferred reporting items for systematic review and meta-analysis (PRISMA) statements [27].

Search strategy

A systematic literature search was conducted by two inde-pendent reviewers (JR and HKV) in PubMed/MEDLINE, Cochrane, and SCOPUS (from inception to January 2019). Randomized Control Trials (RCTs) evaluating the e ects of metformin on biomarkers associated with breast cancer out-comes were considered for inclusion. Boolean search terms (AND, OR, or NOT) were used to create a search strategy, combining search terms related to the exposure (metformin therapy) and outcomes (biomarkers associated with breast cancer: insulin, glucose, CRP, leptin, BMI, cholesterol, Ki-67, and HOMA). We adopted a specific approach when developing the search strategy, without using date or lan-guage restrictions. We report details of the search strategy in the supplementary, Table 1.

Selection criteria

The participant, intervention, comparison, and outcome (PICO) criteria was used to establish study eligibility. Two authors (JR and NM) independently reviewed the abstract of all articles to select eligible studies. Titles and abstracts obtained from each database were stored in Endnote Refer-ence Manager X8© and duplicates were excluded using the Endnote function "remove duplicates". Two investigators (JR and NM) independently reviewed the full texts of rel-evant articles. The following inclusion criteria were used: (1) studies on adult patients (age > 18 years) with breast cancer that reported data on exposure to metformin therapy versus placebo; (2) RCT studies reporting results in the form of mean di erences (MD) with the 95% confidence intervals (95% CI) or reporting sufcient results for these estimates to be derived. Exclusion criteria were as follows: (1) studies comparing the efcacy of drugs not including metformin; (2) other types of cancers; (3) studies not reporting biomarkers

Table 1 Baseline characteristics of included studies in the meta-analysis

Studies	Author	Country	Year	Fol-low up, years	Patients, *n*	Mean age, years	Metformin dose (mg/ day)	Biomarker
1	Patterson RE	USA	2018	24	333	62	1500	Insulin, Glucose, CRP
2	Davis SR	Australia	2018	52	83	56	1700	Insulin, Glucose, HOMA, BMI
3	Sadighi S	Iran	2016	24	45	50	1500	Insulin, Glucose, CRP, HOMA, Ki-67
4	Ko KP	Republic of Korea	2015	24	81	–	500	Insulin, Glucose, BMI, Cholesterol
5	Ko KP	Republic of Korea	2015	24	81	–	1000	Insulin, Glucose, BMI, Cholesterol
6	Goodwin PJ	Canada	2015	24	492	52	850	Insulin, Glucose, CRP, HOMA, Leptin, BMI
7	Kalinsky K	USA	2014	2	66	56	1500	Insulin, Glucose, HOMA, Leptin, BMI, Cholesterol, Ki-67
8	Bonanni B	Italy	2012	4	196	52	1700	Insulin, Glucose, CRP, Cholesterol, Ki-67
9	Hadad S	UK	2011	2	39	63	1000	Insulin, Ki-67

associated with breast cancer; (4) animal studies; (5) non-randomized study designs or designs without a placebo group; (6) conference abstracts, commentaries, case reports, and reviews.

Data extraction

Two reviewers (JR and NM) independently extracted the data using a predefined data extraction form. Discrepan-cies were discussed and resolved by senior author (YZ). The information extracted included author, year of publica-tion, country, geographic location, duration of follow-up, number of patients, mean age (years), metformin dose (mg/ day), and mean and SD of outcome in baseline study and post-intervention.

Quality assessment

Studies included in this review were critically appraised using the Cochrane collaboration's tool for quality assess-ment of randomized control trials [28], which considers the following domains: selection bias (random sequence genera-tion and allocation concealment), performance bias, detec-tion bias, attrition bias, reporting bias, and other sources of bias. Each study was classified as having a low, high, or unclear risk of bias for each domain. A study was considered to be at low risk of bias if they fulfilled three key criteria related to randomization, allocation concealment, and out-come assessor blinding, and each study was rated as having good, fair, or poor quality according to the AHRQ Standards [28]. The quality of evidence for each study was considered to be good quality when all the domains were rated as low and was classified as good quality study.

Statistical analysis

The e ects of metformin therapy on biomarkers associ-ated with breast cancer were measured by weighted mean di erence (WMD) with the 95% CI. When the standard deviation (SD) of the mean di erence for studies was not reported, we calculated the SD using the following for-mula: SD2 baseline + SD2 final − (2 R × SD baseline + SD final) [29]. The random- e ects model (DerSimonian and Laird method) was used to calculate the pooled weighted mean di erence (WMD). Heterogeneity across studies was assessed using the Q test, and the I- squared and an alpha of 0.05 for statistical significance were used. We used Cochrane thresholds recommendation for system-atic reviews [30]. We defined heterogeneity using the fol-lowing criteria: I^2 of 0–30% unimportant heterogeneity, I^2 = 30–60% moderate, 50–90% substantial heterogeneity, and I^2 = 75–100% high. We performed subgroup analysis to identify the probable source of heterogeneity among trials. Duration of intervention (≤ 4 week or > 4 week) was considered as predefined source of heterogeneity. Sensitiv-ity analysis was performed to investigate the e ect of each study on overall analysis. The likelihood of publication bias was determined using Egger's and begg's weighted regression tests and funnel plot. The non-linear potential effects of metformin dosage (mg/day) were examined using fractional polynomial modeling. Meta-regression was used to determine e ect of participant age on inter-vention outcomes. All statistical tests were conducted using the STATA 14 (StataCorp LP, College Station, USA), using a p value of 0.05 for statistical significance.

Results

We retrieved 249 articles using our search strategy (Sup-plemental Fig. 1). After removing duplicates, 197 articles were screened at title and abstract using the study selection criteria. We excluded 179 articles and retrieved 18 poten-tially relevant studies for full-text assessment of eligibility. Eight articles (9 studies) [31–38] fulfilled the inclusion cri-teria and were included in the meta-analysis. Nine articles were excluded for the following reasons: (1) no RCT design (n = 3), (2) no human trial (n = 5), and (3) no placebo (n = 2).

Study characteristics

Characteristics of studies are presented in Table 1. These studies were conducted in the US [35 , 37], Australia [32], Iran [38], Republic of Korea [36], Canada [33], Italy [31], and UK [34]. Most studies were recent publications (between 2011 and 2018). The sample size was 1363 partici-pants, ranging from 39 to 492. Mean dose of the metformin was 1250 mg/day (ranging from 500 to 1700 mg/day). Nine studies investigated the e ect of metformin compare to pla-cebo administered to patients with breast cancer on levels of blood circulating insulin levels [31–38], eight on glu-cose [31–33, 35–38], four on CRP [31, 33, 37, 38], four on HOMA [32, 33, 35 , 38], two on Leptin [33 , 35], five on BMI [32, 33, 35, 36], four on cholesterol [31, 35, 36], and four on Ki-67 [31, 34 , 35 , 38]. Table 2 provides the quality assessment results of the studies.

Risk of bias assessment

Two studies were judged to have fair quality [31, 33] (Table 2), three poor quality [34, 35, 38], and have good quality [32, 36, 37]. Most studies were judged to be of poor quality due to inadequate randomization techniques and blinding of participants as well as assessors.

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Table 2 Quality of the studies

Study name	Selection bias random sequence generation	Selection bias allocation concealment	Reporting bias selective reporting	Other bias other sources of bias	Performance bias blinding	Detection bias blinding	Attrition bias incomplete outcome data	AHRQ Standards
Bonanni, 2012	Unclear	Unclear	Low	Low	Unclear	Unclear	Low	Fair quality
Goodwin, 2015	Unclear	Unclear	Low	Low	Low	Low	Low	Fair quality
Davis, 2018	Low	Low	Low	Low	Low	Low	Low	Good quality
Hadad, 2011	Low	Low	Low	High	High	High	Low	Poor quality
Kalinsky, 2014	Not applicable	Not applicable	Low	High	Not applicable	Not applicable	Low	Poor quality
Ko, 2015	Low	Low	Low	Low	Low	Low	Low	Good quality
Ko, 2015	Low	Low	Low	Low	Low	Low	Low	Good quality
Patterson, 2018	Low	Low	Low	Low	Low	Low	Low	Good quality
Sadighi, 2016	Not applicable	Not applicable	Low	High	Not applicable	Not applicable	Low	Poor quality

Results of meta analysis

Nine studies provided a total of 1363 participants (interven-tion = 665 and control = 698) reported levels of insulin as an outcome measure. We combined the results using random-e ects model and found significant reductions in insulin levels following metformin intervention (WMD: − 0.99 U/ ml, 95% CI − 1.66, − 0.33) (Fig. 1). There was significant heterogeneity among studies ($p = 0.01$, $I^2 = 59.8$).

Eight studies providing a total of 830 participants (inter-vention = 647 and control = 675) reported glucose as an outcome measure. We pooled data using a random-e ects model and showed that there was a significant reduction in blood glucose levels in the metformin group compared with the control group (WMD: − 1.78 ml/dl, 95% CI − 2.96, − 0.60), with significant heterogeneity among studies ($p = 0.09$, $I^2 = 42.9$).

Compared with placebo, metformin intervention was associated with a significant reduction in CRP levels (WMD: − 0.60 mg/l, 95% CI − 0.88, − 0.33) with no indication of heterogeneity among the studies ($p = 0.73$, $I^2 = 0.00$).

For HOMA, the combined e ect size was − 0.45 (95% CI − 0.73, − 0.13) with no indication of sufcient heterogeneity among the studies ($p = 0.20$, $I^2 = 33.9$).

Results from a random-e ects model indicated that met-formin intervention resulted in significant reduction in leptin (WMD: − 2.44 ng/ml, 95% CI − 3.28, − 1.61 with no indi-cation of heterogeneity between studies ($p = 0.61$, $I^2 = 00.0$).

Five studies providing 803 participants (case = 359, and control = 391) reported BMI as an outcome measure. We pooled data using a random-e ects model and showed that BMI reduced in the metformin group compared with the control group (WMD: − 0.55 kg/m^2, 95% CI − 1.00, − 0.11). There was no indication of significant heterogeneity among studies ($p = 0.17$, $I^2 = 41.2$).

We combined results from four studies using a random-e ects model. The results indicated that metformin interven-tion demonstrated a non-significant reduction in cholesterol levels (WMD: − 6.80 mg/dl, 95% CI − 15.23, 1.64) with sig-nificant heterogeneity among the studies ($p = 0.04$, $I^2 = 62.5$).

We also combined results from four numbers of stud-ies using a random-e ects model. For Ki-67, the combined e ect size was − 4.06 (95% CI − 7.59, − 0.54), with sig-nificant heterogeneity between studies ($p = 0.01$, $I^2 = 79.1$).

Subgroup analysis

Results of the subgroup analyses are summarized in Table 3. We stratified studies based on duration of metformin intervention (≤ 4 and > 4 week). The results of the subgroup analyses showed that insulin levels (WMD: − 0.78 U/ml, 95% CI − 1.26, − 0.30), glucose levels (WMD: − 2.39 mg/ dl, 95% CI − 3.73, − 1.04), and BMI (WMD: − 0.58 kg/m^2, 95% CI − 1.09, − 0.08) decreased more significantly when the duration of administering metformin intervention was above 4 weeks compared with trials with less than 4-week duration (see Fig. 1).

We performed subgroup analyses for cholesterol lev-els. The results showed that cholesterol levels (WMD: − 13.03 mg/dl, 95% CI − 19.35, − 6.71) decreased signifi-cantly when metformin intervention duration was less than 4 weeks compared with trials with above 4-week duration (WMD: 1.34 mg/dl, 95% CI − 6.95, 9.63).

We did not perform subgroup analysis for CRP, HOMA, leptin, and Ki- 37, because duration of interventions between studies was similar (see Fig. 1).

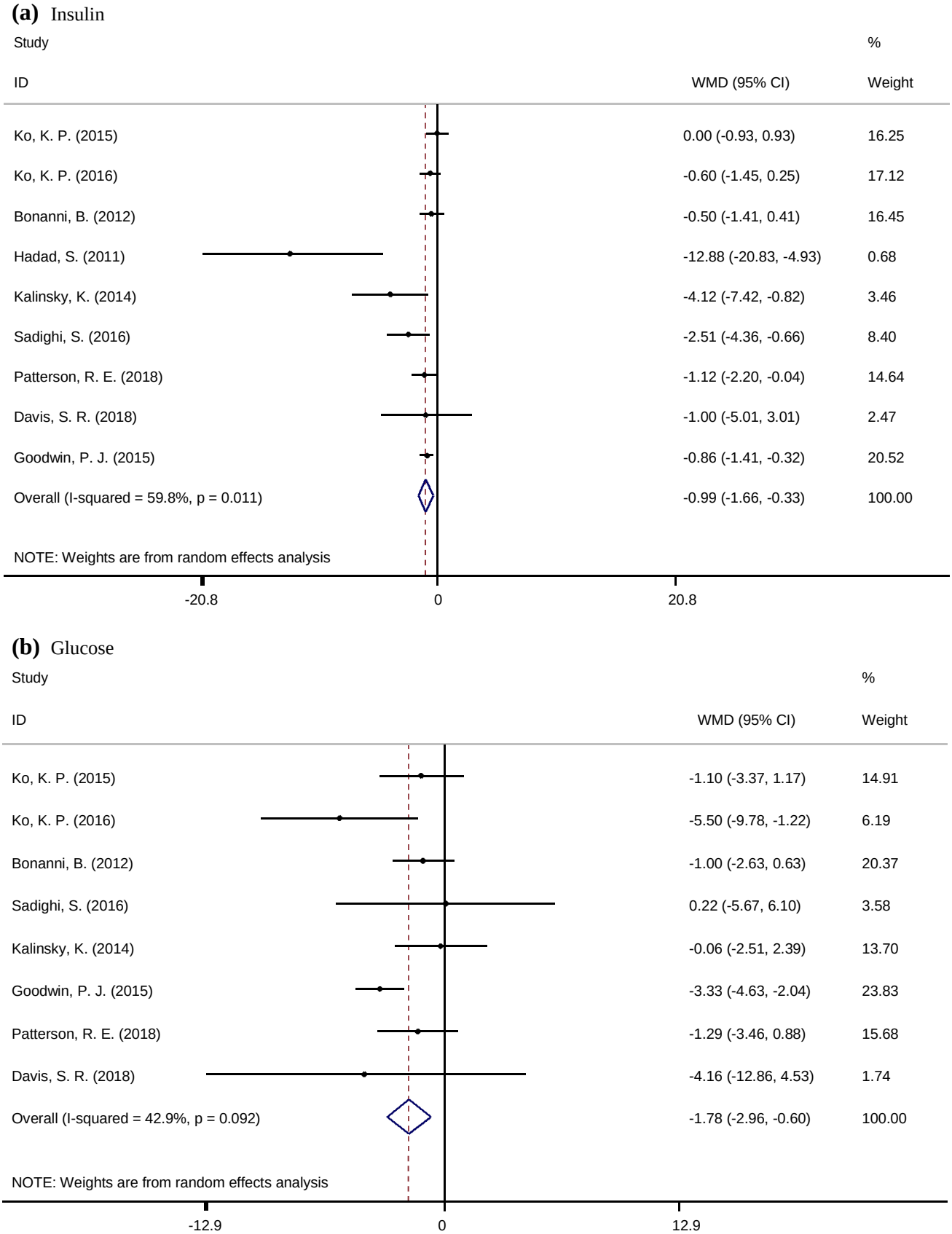

Fig. 1 Forest plot of randomized controlled trials investigating the e ects of Metformin on: **a** insulin; **b** glucose; **c** CRP; **d** HOMA; **e** leptin; **f** BMI; **g** cholesterol; **h** Ki-67

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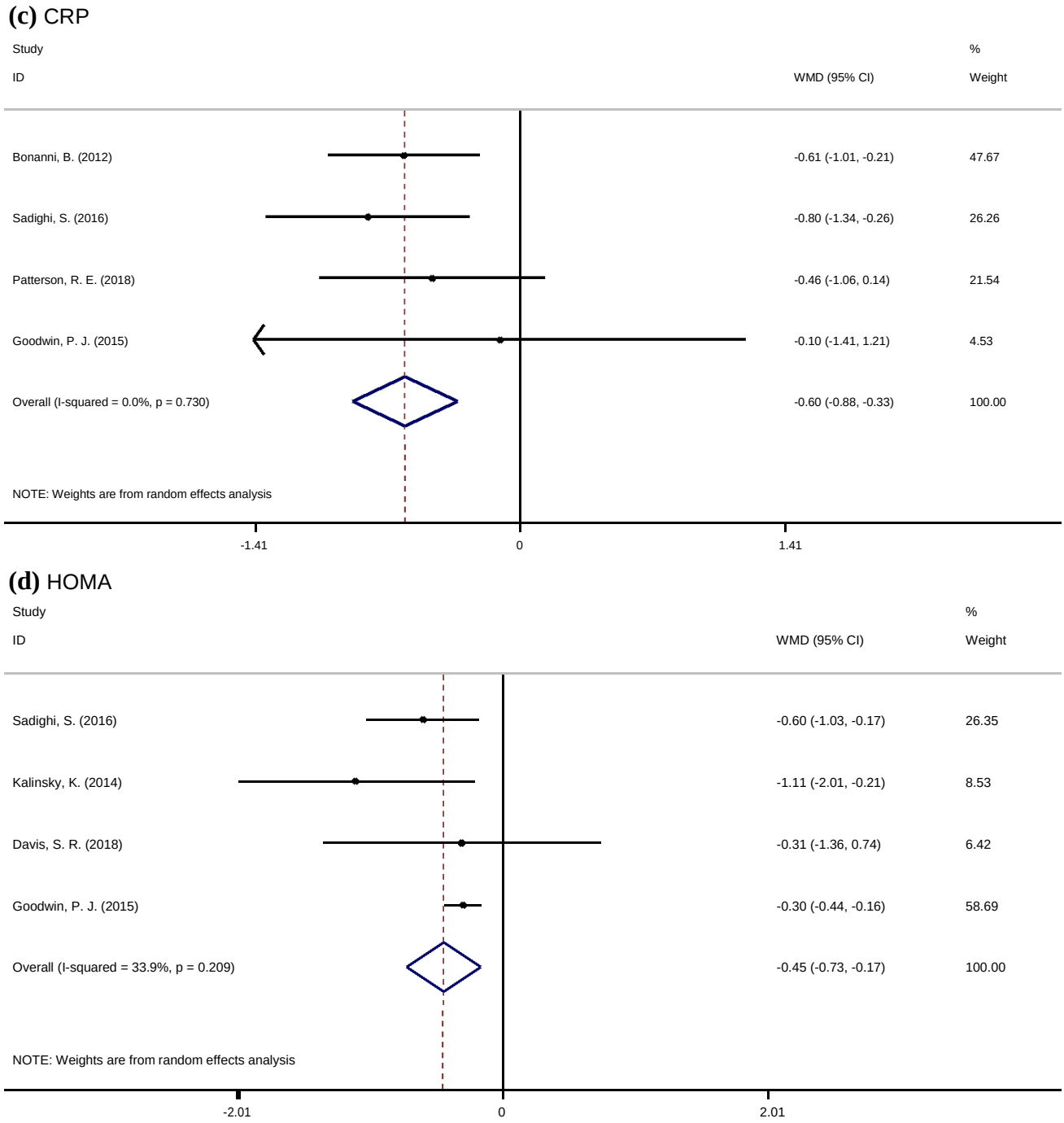

Fig. 1 (continued)

Meta regression and non linear dose–responses

We explored the dose–response relationship between dose of metformin (mg/day) and outcome. We found a non-linear fashion between biomarkers and dose of metformin Insulin ($P_{\text{nonlinearity}} = 0.22$), glucose ($P_{\text{nonlinearity}} = 0.18$),

CRP ($P_{nonlinearity}$ = 0.82), HOMA ($P_{nonlinearity}$ = 0.41), BMI ($P_{nonlinearity}$ = 0.39), cholesterol ($P_{nonlinearity}$ = 0.34), and Ki-67 ($P_{nonlinearity}$ = 0.30) (Supplemental Fig. 2).

We used meta-regression analysis to examine the vari-ation in treatment e ect of metformin based on age of participants. The results of the meta-regression suggested that participants' age was not a significant source of clinical heterogeneity (Supplemental Fig. 3).

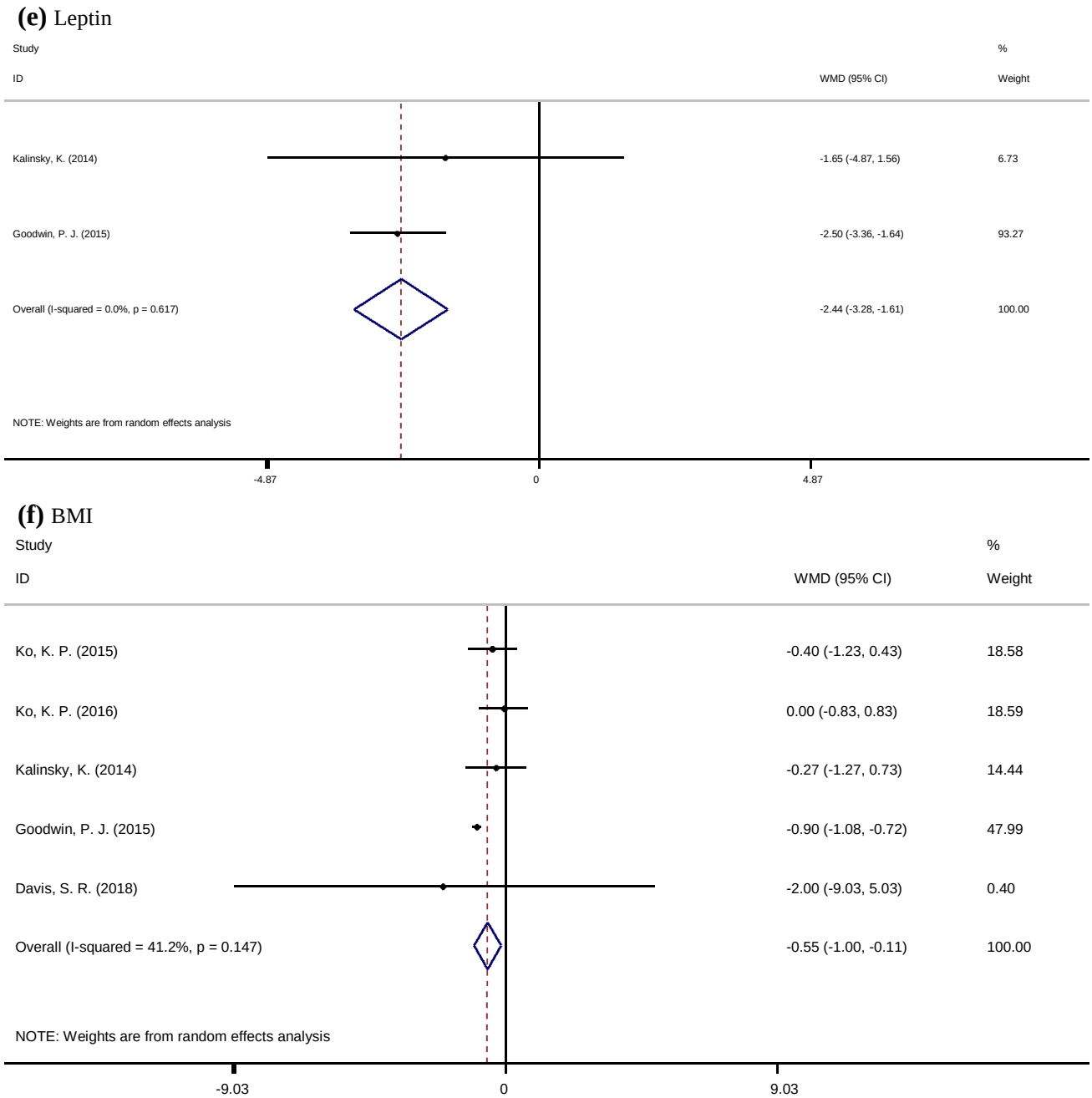

Fig. 1 (continued)

Publication bias and sensitivity analysis

The Egger's and Begg's tests did not show any publication bias for BMI (p = 0.20, p = 0.97), cholesterol (p = 0.38, p = 0.49), CRP (p = 0.36, p = 0.17), glucose (p = 0.79, p = 0.98), HOMA (p = 0.23, p = 0.98), Ki-67 (p = 0.56,

p = 1.00), and leptin (p = 1.00, p = 0.31), respectively (Supplemental Fig. 4). However, there was a significant publication bias for insulin (p = 0.04, p = 0.09). ‘Trim-and-fill’ method used for adjusting publication bias, but did not show potentially missing studies for the meta-analyses of insulin. The results of the sensitivity analysis did not show significant di erences beyond the limits of 95% CI between calculated SESs for metformin intervention stud-ies (Supplemental Fig. 5).

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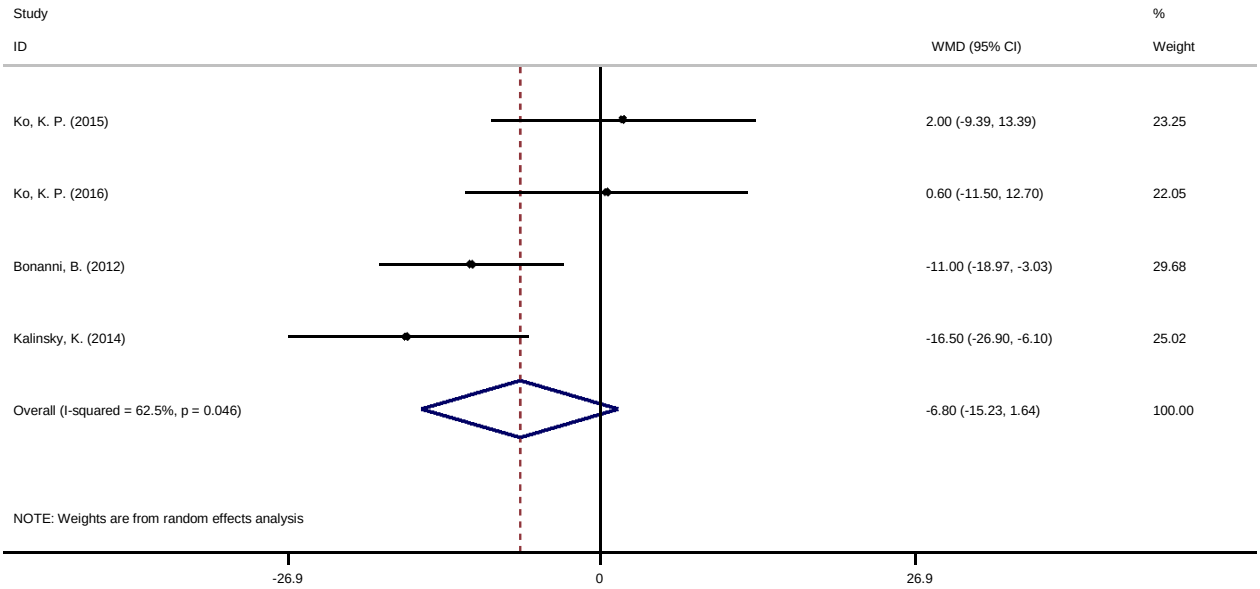
(g) Cholesterol



(h) Ki-67

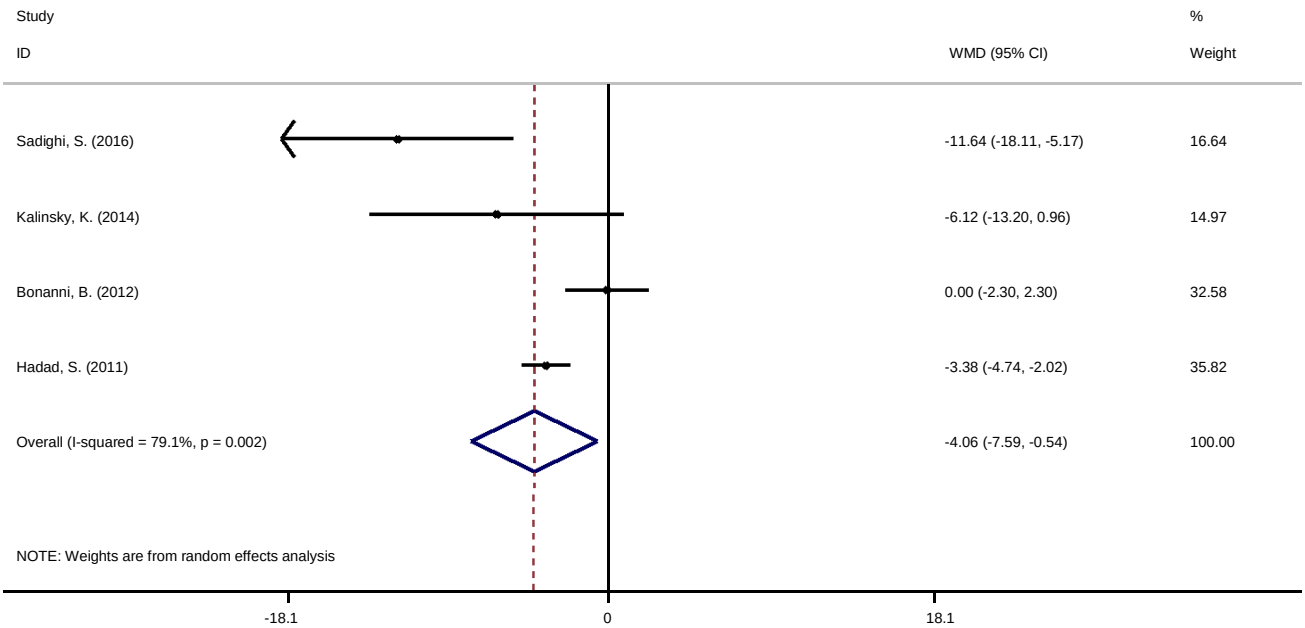


Fig. 1 (continued)

Discussion

This systematic review aimed to estimate the e ect of met-formin intervention on biomarkers associated with breast cancer outcomes. To the best of our knowledge, this is the first systematic review of randomized -controlled tri-als investigating the e ects of metformin on biomarkers among diabetic patients with breast cancer. The results demonstrated that diabetic patients with breast cancer showed significant reductions in insulin, fasting glucose, CRP, HOMA, leptin, BMI, and Ki- 67 when receiving met-formin compared with the control group.

There was evidence from nine RCTs investigating the effects of metformin therapy among breast cancer patients The results showed that metformin significantly reduces the level of circulating blood insulin. Metformin reduces the levels of blood circulating insulin by improving insu-lin sensitivity, increasing insulin receptor tyrosine kinase activity, enhancing glycogen synthesis, decreasing hepatic gluconeogenesis, and increasing the activity of GLUT4 glucose transporters [39, 40].

We pooled data from eight RCTs that administered metformin and observed a reduction in blood glucose con-centration through its direct hypoglycemic e ect such as enhancing glycogen synthesis and decreasing hepatic gluco-neogenesis by enhancing the LKB1/AMP-activated protein kinase (LKB1/AMPK) pathway [40]. Chronic inflammation negatively impacts on the outcomes of breast cancer by the inactivation of tumor suppressor genes and stimulating angi-ogenesis and antigenic factor production [41–43]. When data were pooled from four studies [26, 31, 33, 38], metformin showed a beneficial e ect of reducing CRP, a marker of chronic inflammation.

Metformin demonstrated no difference in effects on cholesterol levels for the intervention group compared to the control group. However, this finding may have been due to heterogeneity between groups of patients included in the meta-analysis. A subgroup analyses of the results by duration of intervention (< 4 weeks or > 4 weeks) showed a significant decrease of cholesterol levels in studies that conducted less than 4 weeks. This is similar to results of a systematic review reported by Wul elé and colleagues. The authors investigated the e ect of metformin on blood pres-sure, plasma cholesterol, and triglycerides in type 2 diabetes mellitus patients, and showed that total cholesterol reduced when duration of intervention by metformin was less than 12 weeks compared with longer interventions [44].

We performed subgroup analysis to evaluate the impact of duration of metformin on insulin concentration, blood glucose, and BMI. Significant improvements were observed at > 4 weeks of metformin therapy. Reductions in the weighted mean di erence of blood glucose were observed with comparisons of < 4 weeks and > 4 weeks' duration of metformin therapy, favoring interventions administered for longer durations. Participants receiving care for > 4 weeks showed glucose reductions which was twice as high com-pared to metformin therapy of lesser duration. This pattern of improvement at > 4 weeks was observed for HOMA, lep-tin, BMI, and Ki-67. This suggests that treatment for longer duration was associated with significant improvement in clinical outcome. However, for cholesterol concentration, better outcomes indicated by WMD: − 13.03 mg/dl (95% CI − 19.35, − 6.71) were observed with the administration of metformin for a shorter duration of < 4 weeks.

Cancer cells have an underlying pathophysiology of increasing the level of blood circulating insulin and over stimulating signaling pathways of IGF, leading to exces-sive growth of cancerous cells [45]. Metformin demon-strates its therapeutic e ect through its direct and indirect mechanisms, reducing insulin resistance and providing optimum glycemic control in breast cancer patients [46]. Metformin acts by inhibiting the mTOR pathway; activat-ing autonomous AMP-activated protein kinase (AMPK), leading to phosphorylation of tuberous sclerosis complex protein 2. This leads to a decrease in protein synthesis and cell growth of cells [47–50]. Metformin reduces the levels of HOMA and leptin, higher concentration of which is associated with poor outcomes. Leptins are believed to act on the OB-2 receptor leading to activation of STAT signaling pathway and other pathways involving Ras–Raf MEK signaling. Metformin indirectly reduces the levels of leptins, inhibiting OB- 2 receptor pathway. Other suggested mechanisms of action through which Metformin exerts its indirect anti-cancerous e ect are by increasing TUNEL, a marker for apoptosis and reduces Ki-67, a marker of prolif-eration [47, 51]. Furthermore, metformin demonstrates its weight loss e ect by improving mediators of insulin resist-ance, regulating fat oxidation, decreasing hepatic glucose output, and inhibiting gluconeogenesis. This improves blood glucose control, decreasing food consumption and intestinal glucose absorption [52–54].

There is a conflicting evidence from the previous reviews evaluating the e ects of metformin among dia-betic breast cancer patients. A meta- analysis of observa-tional studies by Nandana et al. [18] assessed Metformin and breast cancer risk. The authors reported a significant protective e ect of metformin on breast cancer risk among diabetic women [OR 0.83 (95% CI 0.71–0.97)] [18]. This is supported by findings by Pamela and colleagues where the intervention study showed improvement in insulin lev-els, HOMA, leptins, and CRPs with metformin therapy [55]. This is, however, inconsistent with results from a similar meta -analysis of observational studies conducted by Tang and colleagues assessing the e ect of metformin in breast cancer incidence and mortality. The authors found no association between metformin therapy among diabetic breast cancer patients and concluded that there is need for evidence from more rigorous study designs to investigate the e ects of metformin on the survival rates in breast cancer [56]. Similarly, Bonanni et al. reported no significant decrease in Ki- 67 among patients receiv-ing metformin [31]. This is in contrast to findings of our meta-analysis suggesting beneficial anticancer e ects of metformin among diabetic breast cancer patients. Overall, the findings of this review showed that metformin reduces the insulin concentration improving glycemic control, and reduces levels of HOMA -IR, Leptin, Ki-67, and CRP, which improves outcome of increased survivals among breast cancer patients.

This meta-analysis has several strengths and limitations. The main strength of the study is the use of randomized-controlled trials as the study design of choice to investigate the association between the intervention and outcome. We explored sources of heterogeneity among studies included in this meta-analysis, using subgroup analysis based on dura-tion of interventions, age of participants and dose of met-formin, and performed sensitivity analysis.

However, there were some limitations to the study. Although we conducted a comprehensive search to reduce bias, we did not perform hand searching of journals; hence, some studies may have been missed. Although we evalu-ated our studies using a robust quality assessment tool, these judgements were qualitative and inter-rater reliabilities were not explored. Another limitation of this study is the small number of studies available for subgroup analysis of the e ect of metformin on most biomarkers. Larger studies with adequate sample size are needed to inform definitive conclu-sions. We were reliant on data from significantly heterogene-ous studies that were found to be at high risk of bias.

Conclusions

The results of this showed that metformin intervention sig-nificantly reduces insulin, fasting glucose, CRP, HOMA-IR, leptin, BMI, and Ki- 67 levels among breast cancer patients. Greater reductions in insulin, glucose, and BMI where observed when the duration of intervention was ≥ 4 weeks. However, changes in cholesterol levels were not observed and quality of the evidence from this review was poor. We suggest that cautions were interpreting the findings of this review as most studies demonstrated high risk of bias. We recommend that evidence from robust studies with long-term follow-up is needed to make definitive clinical recommendations.

Variables	Duration (week)		All (–)
	≤ 4	> 4	
Insulin			
Number of studies	3	6	9
Weighted mean di erence (WMD)	− 4.35	− 0.78	− 0.99
95% CI	− 9.28, 0.57	− 1.26, − 0.30	− 1.66, − 0.33
p-heterogeneity	0.01	0.24	0.01
Glucose			
Number of studies	2	6	8
Weighted mean di erence (WMD)	− 0.71	− 2.39	− 1.78
95% CI	− 2.07, 0.64	− 3.73, − 1.04	− 2.96, − 0.60
p-heterogeneity	0.53	0.21	0.09
CRP			
Number of studies	1	3	4
Weighted mean di erence (WMD)	− 0.61	− 0.60	− 0.60
95% CI	− 1.01, − 0.20	− 0.98, − 0.21	− 0.88, − 0.33
p-heterogeneity	–	0.52	0.73
HOMA			
Number of studies	1	3	4
Weighted mean di erence (WMD)	− 1.11	− 0.33	− 0.45
95% CI	− 2.01, − 0.21	− 0.46, − 0.19	− 0.73, − 0.17
p-heterogeneity	–	0.42	0.20
Leptin			
Number of studies	0	2	2
Weighted mean di erence (WMD)	–	2.20	− 2.44
95% CI	–	1.60–3.04	− 3.28, − 1.61
p-heterogeneity	–	0.540	0.61
BMI			
Number of studies	1	4	5
Weighted mean di erence (WMD)	− 0.27	− 0.58	− 0.55
95% CI	− 1.26, 0.72	− 1.09, − 0.08	− 1.00, − 0.11
p-heterogeneity	–	0.13	0.14
Cholesterol			
Number of studies	2	2	4
Weighted mean di erence (WMD)	− 13.03	1.34	− 6.80
95% CI	− 19.35, − 6.71	− 6.95, 9.63	− 15.23, 1.64
p-heterogeneity	0.41	0.86	0.04
Ki-37			
Number of studies	3	1	4
Weighted mean di erence (WMD)	− 2.39	− 11.64	− 4.06
95% CI	− 5.33, 0.54	− 18.10, − 5.17	− 7.59, − 0.54
p-heterogeneity	0.02	–	0.01

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