RESEARCH

Insulin-like growth factor role in determining the anti-cancer effect of metformin: RCT in prostate cancer patients

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Abstract

Objective: Androgen deprivation therapy (ADT), a principal therapy in patients with prostate cancer, is associated with the development of obesity, insulin resistance, and hyperinsulinemia. Recent evidence indicates that metformin may slow cancer progression and improves survival in prostate cancer patients, but the mechanism is not well understood. Circulating insulin-like growth factors (IGFs) are bound to high-affinity binding proteins, which not only modulate the bioavailability and signalling of IGFs but also have independent actions on cell growth and survival. The aim of this study was to investigate whether metformin modulates IGFs, IGF-binding proteins (IGFBPs), and the pregnancy-associated plasma protein A (PAPP-A) – stanniocalcin 2 (STC2) axis.

Design and methods: In a blinded, randomised, cross-over design, 15 patients with prostate cancer on stable ADT received metformin and placebo treatment for 6 weeks each. Glucose metabolism along with circulating IGFs and IGFBPs was assessed.

Results: Metformin significantly reduced the homeostasis model assessment as an index of insulin resistance (HOMA IR) and hepatic insulin resistance. Metformin also reduced circulating IGF-2 ($P < 0.05$) and IGFBP-3 ($P < 0.01$) but increased IGF bioactivity ($P < 0.05$). At baseline, IGF-2 correlated significantly with the hepatic insulin resistance ($r^2 = 0.28$, $P < 0.05$). PAPP-A remained unchanged but STC2 declined significantly ($P < 0.05$) following metformin administration. During metformin treatment, change in HOMA IR correlated with the change in STC2 ($r^2 = 0.35$, $P < 0.05$).

Conclusion: Metformin administration alters many components of the circulating IGF system, either directly or indirectly via improved insulin sensitivity. Reduction in IGF-2 and STC2 may provide a novel mechanism for a potential metformin-induced antineoplastic effect.

Key Words

▶ IGFBP-3
▶ bioactive IGF-1
▶ pregnancy-associated plasma protein-A
▶ stanniocalcin 2
▶ insulin resistance

Introduction

Prostate cancer is the most common solid organ cancer in men and androgen deprivation therapy (ADT) is a principal therapy. While ADT improves cancer symptoms and survival in prostate cancer patients, because of the induced hypogonadism, it is associated with the development of obesity, insulin resistance, hyperinsulinemia, and increased cardiovascular disease risk (1, 2). The metabolic syndrome is present in >50% of men receiving long-term ADT compared to 20% of matched controls (3). Insulin resistance is increased by more than 30% as early as 3 months after the initiation of ADT (4), and the risk of new-onset diabetes is increased four- to five-fold in the first year of treatment (5). These conditions are known to worsen cancer risk and prognosis, partly due to the carcinogenic effects of hyperinsulinemia (6). Thus, in prostate cancer patients on ADT, there are high rates of obesity, hyperinsulinemia, and diabetes development, which may be associated with a poorer cancer prognosis (1, 2).

Chronic hyperinsulinemia may stimulate carcinogenesis either directly through the insulin receptor or indirectly through insulin-like growth factor receptors (IGF-1Rs). Recent evidence indicates that the anti-diabetic drug metformin may slow cancer progression and improve survival in prostate cancer patients (7, 8, 9). Diabetic patients treated with metformin have a significantly reduced risk of cancer, with meta-analysis reporting metformin to be associated with reduction in overall cancer incidence by 31% and cancer mortality by 34% (10), including reduced prostate cancer-specific mortality (7, 8). There are currently several studies underway that evaluate the potential clinical benefit of the addition of metformin to standard therapy for advanced prostate cancer (STAMPEDE trial (NCT00268476), MAST study (NCT01864096), and PRIME study (NCT03031821)). A recent randomised, controlled trial indicated that metformin improves castration-resistant prostate cancer-free survival in patients with prostate cancer (11). To date, however, there is a paucity of studies that explore the mechanisms of the potential anticancer effect of metformin in patients with prostate cancer.

The mechanism of action of metformin remains poorly understood (12). Metformin has been shown to inhibit mitochondrial glycerophosphate dehydrogenase, downregulate androgen receptors, activate AMPK, and suppress the PI3K/AKT pathway which results in the inhibition of the mTOR pathway (13, 14, 15, 16). There is increasing evidence that metformin, by modulating insulin-like growth factors (IGFs) and IGF-binding proteins (IGFBPs), may inhibit cancer proliferation and spread. Recent studies show that metformin inhibits androgen-induced IGF-1R gene transcription in prostate cancer cells (17). In pancreatic cancer, metformin reduces proliferation through the activation of AMPK and inhibition of IGF-1R signalling (13).

The IGFs are mitogenic peptides involved in the regulation of cell proliferation (18). Circulating IGFs originate primarily from the liver; however, they are also produced locally by prostatic stromal cells in response to androgen stimulation, thus increasing epithelial cell proliferation. Epidemiological studies have established a link between high circulating IGF-1 and a greater risk of advanced prostate cancer (19), in which the IGF-1R signalling pathway is upregulated (20). IGFs circulate bound to high-affinity binding proteins, which not only modulate the bioavailability and signalling of IGFs but also have IGF-independent actions on cell growth and survival (21). Therefore, IGF and IGFBPs signalling may drive cancer development and progression (22). IGFBP-2 has been shown to stimulate cancer growth and invasion, whereas IGFBP-3 may exert protective antineoplastic effects (23, 24). Thus, IGFBPs have additional IGF-1-independent effects on cancer.

In determining the role of IGFBPs in cancer development, it is important to consider not only a change in their concentration but also a change in their proteolytic cleavage and hence IGF-binding capacity. There are several factors that regulate IGFBPs cleavage, with pregnancy-associated plasma protein-A (PAPP-A) recently sparking great interest in cancer pathogenesis (25). PAPP-A is a metalloprotease that cleaves several IGFBPs, with IGFBP-4 being the key substrate. The cleavage allows the IGFs to separate from the IGFBPs, which results in an increase in IGF bioactivity (26, 27). Stanniocalcin 2 (STC2) has oncogenic properties (28) and is an inhibitor of PAPP-A reducing its IGFBP-4 proteolytic activity (29). Therefore, STC2 is expected to reduce IGF bioactivity, inhibiting cancer growth. However, many studies show that STC2 has oncogenic properties, many cancers overexpress STC2 and an increased STC2 expression correlates with a poorer prognosis (30). These observations appear to conflict with the role of STC2 as an inhibitor of PAPP-A. However, since STC2 was not previously connected to PAPP-A or the IGF system, they have mostly been studied separately, and the link between STC2 and cancer warrants further investigations. This also illustrates the complexity of the IGF-axis; the activity of the IGFs depends on an intimate relationship between the various components, as further discussed in a recent review (25). Thus, IGFBPs and IGF

bioactivity may be modulated via the PAPP-A/STC2 pathway that plays a major role in cancer biology.

There is a paucity of studies that explore the effects of metformin on IGFs/IGFBPs in patients with prostate cancer. Animal studies have shown that metformin reduces total IGF-1 levels and prostate cancer mortality, and a further study in humans reported a decrease in circulating IGF-1 following 12 weeks of metformin treatment (31, 32). In addition, metformin has been reported to alter serum concentrations of the IGFBPs (33, 34, 35) and this makes it difficult to predict the overall impact of metformin on the circulating IGF bioactivity. To the best of our knowledge, there are no studies in prostate cancer that have comprehensively investigated metformin effects on IGFs, IGF bioactivity, IGFBPs, and IGFBP activity modulators. We hypothesise that metformin induces its antineoplastic effect in prostate cancer patients by modulating IGFs and IGFBPs.

Methods

This was a blinded, randomised, controlled cross-over study of metformin treatment in patients with prostate cancer. Men with prostate cancer who were on stable ADT for at least last 6 months were invited to participate in this study. Patients were recruited from the Crown Princess Mary Cancer Centre, Westmead Hospital, and the Blacktown Cancer and Haematology Centre, Blacktown Hospital, Australia. Inclusion criteria were men aged between 50 and 80 years with histologically confirmed prostate cancer of early (localised prostate cancer disease without metastases) or advanced-stage prostate cancer (metastatic prostate cancer with bone involvement only, as patients with visceral metastases tend to have a more aggressive course with a higher chance of progression during the study period and these patients were excluded to ensure the effect of metformin could be tested), Eastern Cooperative Oncology Group (ECOG) 0–1 performance status, and on stable treatment with ADT with GnRH analogues for more than 6 months. In our clinics, patients with non-metastatic disease routinely receive ADT as concurrent/adjuvant therapy, in combination with prostate radiation therapy for high-risk prostate cancer.

Exclusion criteria were visceral metastases, castrate-resistant prostate cancer (PSA progression defined as at least 3 PSA rises, measured on three successive occasions ≥ 1 week apart after hormonal treatment), history of confirmed type 1 or type 2 diabetes mellitus or a positive test during screening by an oral glucose tolerance test (OGTT), current or prior use of metformin or other anti-diabetic drugs

within the last year, known hypersensitivity or allergy to metformin or any of its excipients, hypothalamic or pituitary disorders, other forms of malignancies excluding prostate cancer, renal (eGFR < 60 mL/min/1.73 m^2) or hepatic impairment (bilirubin ≥ 1.5× upper limit normal, ALT and ALP ≥ 2.5× upper limit normal), history of lactic acidosis, cardiac or respiratory insufficiency, alcohol abuse, severe infections that are likely to increase the risk of lactic acidosis, and any medications known to cause interference with the endocrine system (excluding ADT). This study was approved by the Western Sydney Local Health District Human Research Ethics Committee. All participants gave informed written consent. The study was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12615000778583).

Experimental design

Fifteen patients were randomised to receive either metformin or placebo first using a computer random assignment programme. Each treatment was for 6 weeks. Study endpoints were assessed at baseline, after 6 weeks of treatment with metformin and after 6 weeks of placebo, in randomised order. The dose of metformin was 500 mg daily for the first week, increased to 500 mg twice daily for the second week, to a maximum dose of 1500 mg daily from the third week. Safety and drug-related toxicities were evaluated on scheduled visits during trial treatment and weekly phone calls. One patient developed gastrointestinal side-effects during metformin treatment which required the metformin dose to be increased at a slower pace, achieving target dose at week 4. Another patient developed an ischemic stroke during the placebo treatment phase (after metformin treatment had been completed) and was subsequently withdrawn from the study.

Study endpoints

The primary objective of this study of metformin treatment was to assess a change in the IGF/IGFBP system: IGF-1 and -2, IGF bioactivity, IGFBP-1 to -3, and IGFBP activity modulators PAPP-A and STC2.

Glucose and insulin indices

Glucose metabolism was assessed using the OGTT. Blood glucose and insulin concentrations were measured at baseline and after a 75 g glucose load at 30, 60, 90, and 120 min. Hepatic insulin resistance (the product of total area under the curve for glucose and insulin during the first

30 min) was calculated (36). Homeostasis model assessment as an index of insulin resistance (HOMA IR) was calculated, and overall glucose metabolism was estimated as the incremental glucose and insulin area under the curve above fasting over 120 min.

Body composition and bone mineral density

Lean body mass (LBM) and total and regional fat mass (FM) were assessed by dual x-ray absorptiometry (DXA; GE Healthcare Lunar Prodigy Pro) and Bioeletrical impedance spectroscopy (BIS) using the ImpediMed Ltd SFB7 analyser (ImpediMed Ltd Qld, Pinkenba, Australia) (37). Change in body cell mass (BCM), a functional component of LBM, was estimated by subtracting extracellular water (ECW) from LBM. Vertebral and hip bone mineral density was also measured by DXA at baseline.

Physical activity

Patients were asked to complete an exercise diary for 1 week prior to each visit and to specify the number of hours of light, moderate, or intense physical activity.

Diet

At each visit, patient diet patterns were assessed by a 24-h recall questionnaire using FoodWorks (Xyris Software Pty Ltd, Brisbane, Australia).

Resting energy expenditure and substrate oxidation rates

Carbohydrate and lipid oxidation, as well as resting energy expenditure (REE), were quantified by indirect calorimetry using a metabolic monitor (ParvoMedics, Sandy, Utah) for 20-min periods. The amount of oxygen consumed and carbon dioxide produced is measured, from which substrate oxidation rates are derived. This was done at baseline (after an overnight fast) and 30 and 90 min after the 75 g glucose load.

Bioactive IGF

IGF bioactivity was determined by an in-house kinase receptor activation (KIRA) assay as originally described (38) with modifications (39). In brief, human embryonic kidney HEK 293 cells transfected with the human IGF-1R cDNA were stimulated with patient serum to measure the ability of serum IGF-1 and IGF-2 to activate the IGF-IR *in vitro*. A serial dilution of rhIGF-1 (WHO 02/254) was used as a calibrator. The binding of IGFs to the IGF-IR and subsequent receptor tyrosine auto-phosphorylation

was detected by an anti-phosphotyrosine antibody and quantified by a commercial phospho-IGF-IR ELISA (R&D Systems; Cat# DYC 1770E). The assay signal is referred to as 'IGF bioactivity', because the IGF-IR can be activated by IGF-1 as well as IGF-2 to a lesser extent. In our hands, IGF-2 crossreacts with the IGF-1R with a potency being 12% of that of IGF-1, whereas insulin has a negligible cross-reactivity (<1%) (38). The limit of detection was <0.08 µg/L. Intra- and inter-assay CVs were 12 and 20%, respectively.

Biochemical investigations

Blood samples were taken at three time points (baseline, 6, and 12 weeks). At each visit, fasting blood samples were taken and stored at –80°C for analysis. All samples for any individual were measured in the same assay run for each analyte. Serum glucose and insulin were measured using commercial assays. Serum total IGF-1 and IGFBP-3 levels were measured using an IDS-iSYS Multi-Discipline Automated Analyser (Immunodiagnostic Systems Nordic SA, Denmark) as previously published (40, 41). Limit of detection for total IGF-1 and IGFBP-3 was 4.4 and 50 ng/mL, respectively. IGF-2 was measured as previously described (42, 43). In brief, IGF-2 was measured by an in-house time-resolved immunofluorometric assay, using the international IGF-2 standard (WHO 96/538, NIBSC) as a calibrator. Anti-IGF-2 antibody (#05-166 clone S1F2, Merck Millipore) was used for coating and for detection, and an anti-IGF-2 antibody (# I-7276, Sigma) was directly tagged with Europium according to the manufacturer's instructions (Perkin Elmer Life Sciences) was applied. Intra-assay and inter-assay CVs were 5 and 10%, respectively. IGFBP-1 and -2 were measured by in-house immunoassays, with intra- and inter-assay CVs, respectively, of 8 and 7% for IGFBP-1 and 5 and 12% for IGFBP-2 (39). Serum PAPP-A and STC2 levels were determined by commercial ELISAs (AnshLabs, Texas, USA) as recently described (44).

Statistical analysis

Data were logarithmically transformed for analysis if not normally distributed. The statistical analysis consisted of a paired *t*-test of change in endpoint and a linear regression analysis was used to investigate associations. To account for the lack of a washout between the placebo and metformin treatment, the effect of sequence of treatment was also sought by unpaired *t*-test. Results are expressed as mean ± S.E.M. and a *P* value <0.05 was considered significant. All analysis was conducted using SPSS statistics v22 (IBM corporation).

Results

Baseline characteristics are shown in Table 1.

There was no significant difference in magnitude of any effect according to the order of treatment.

There was no significant change in plasma concentration of PSA during the study (Table 2). There was no significant change in weight compared to baseline. However, the difference between the treatment periods was statistically significant with a 0.9 ± 0.4 kg lower weight gained following metformin compared to placebo administration ($P < 0.05$; Table 2). This change in weight was explained by a change in fat mass, with a 1.1 ± 0.4 kg lower fat mass gain following metformin compared to placebo ($P < 0.05$). Similarly, truncal fat mass differed between the treatment periods, with a 0.9 ± 0.3 kg less gain in truncal fat during metformin treatment ($P < 0.05$). Carbohydrate oxidation rate measured 30 min after a glucose load was significantly higher during metformin treatment with a group difference of 31 ± 14 mg/min when compared to placebo treatment ($P < 0.05$). However, no significant change in resting energy expenditure or fat oxidation rates was noted (Table 2).

Table 1 Baseline characteristics.

Variable	
Age (years)	70.3 ± 1.6
Weight (kg)	90.3 ± 3.4
BMI kg/m^2	31.1 ± 1.1
SBP (mmHg)	139 ± 5.1
DBP (mmHg)	68 ± 2.6
Gleason score	
7 (*n*)	3
8 (*n*)	4
9 (*n*)	8
Cancer staging	
Localised (*n*)	3
Biochemical recurrence (*n*)	9
Metastatic (*n*)	3
PSA (ng/mL)	0.4 ± 0.3
Lean body mass (kg)	51.3 ± 1.5
LBM (% body weight)	57.6 ± 1.4
Fat mass (kg)	35.2 ± 2.2
Extracellular water (L)	19.4 ± 0.7
BCM (kg)	42.1 ± 3.3
Glucose (mmol/L, fasting	5.1 ± 0.1
Insulin (µU/mL), fasting	13.9 ± 1.4
Energy consumption/day (kJ)	8752 ± 978
Light physical activity (h/day)	4.9 ± 1.0
Moderate physical activity (h/day)	3.2 ± 1.4
High physical activity (h/day)	0.2 ± 0.1

Data are presented as mean ± s.e.m.
BCM, body cell mass; DBP, diastolic blood pressure; LBM, lean body mass; LH, luteinising hormone; *n*, number of patients; PSA, prostate-specific antigen; SBP, systolic blood pressure.

There were no significant changes in circulating glucose or insulin, measured in a fasting state or after the glucose load. At baseline, 11 out of 15 patients had HOMA IR > 1.9, indicating the presence of insulin resistance. HOMA IR and hepatic insulin resistance fell significantly ($P < 0.05$) during metformin treatment compared to baseline (Table 2). There was a significant, positive association between HOMA IR and hepatic insulin resistance at baseline ($r^2 = 0.57$, $P = 0.001$).

There was no significant change in circulating IGF-1, whereas IGF bioactivity increased significantly during metformin treatment ($P < 0.05$). Circulating IGF-2 fell during metformin treatment, but the change did not reach statistical significance ($P = 0.07$). However, upon exclusion of one outlier with double levels of IGF-2 compared to rest of the patients, there was a significant reduction in IGF-2 by 29 ± 12 ng/mL during metformin treatment compared to baseline ($P = 0.03$; Fig. 1A). The individual data of a change in IGF-2 are shown in Supplementary Fig. 1 (see section on supplementary materials given at the end of this article). At baseline, IGF-2 correlated significantly with the hepatic insulin resistance ($r^2 = 0.28$, $p = 0.04$; Fig. 2A). Serum IGFBP-3 fell significantly during metformin treatment, with an average difference of 290 ng/mL ($P < 0.01$). The change in IGFBP-3 during metformin treatment explained only 16% of the change in IGF bioactivity, which was not statistically significant ($P = 0.13$). No changes in circulating IGFBP-1 and IGFBP-2 were noted.

There was a significant reduction in circulating STC2 during metformin treatment ($P < 0.05$, Fig. 1B), whereas PAPP-A remained unchanged. During metformin treatment, change in HOMA IR correlated with the change in STC2 ($r^2 = 0.35$, $P = 0.02$; Fig. 2B). The individual data of a change in STC2 are shown in Supplementary Fig. 2.

Discussion

This study showed that metformin treatment in prostate cancer patients significantly improves insulin resistance, particularly hepatic insulin resistance. There was a reduction in IGF-2 and IGFBP-3, whereas IGF-1 concentration remained unchanged, while IGF bioactivity increased during metformin treatment. Circulating STC2 fell significantly during metformin treatment and correlated significantly with HOMA IR. Thus, we were able to demonstrate that metformin modifies the IGF/IGFBP system and affects circulating STC2 thereby providing insight into the possible mechanisms of metformin action in prostate cancer patients.

Table 2   The effect of metformin on study variables.

Variable	Baseline	Change vs baseline metformin (*n* = 15)	Change vs baseline placebo (*n* = 14)	*P* value
Weight (kg)	90.3 ± 3.4	−0.44 ± 0.3	0.54 ± 0.4	**0.02**
Fat mass (kg)	35.2 ± 2.2	−0.3 ± 0.5	0.8 ± 0.3	**0.03**
Fat mass trunk (kg)	20.2 ± 1.1	−0.08 ± 0.5	0.8 ± 0.5	**0.02**
LBM (kg)	63.1 ± 1.5	−0.3 ± 0.5	−0.4 ± 0.4	0.7
REE (kcal/day)	1595 ± 61	−88 ± 57	−80 ± 52	0.71
Cox (mg/day)	55.1 ± 18.4	12.3 ± 25.5	−1.2 ± 18	0.72
Change in Cox 30 min after glucose load	61 ± 6.5	20.3 ± 11.8	−11.3 ± 5.8	**0.047**
Glucose fasting (mmol/L)	5.2 ± 0.1	−0.02 ± 0.07	− 0.03 ± 0.09	0.75
Glucose AUC (nmol × 120 min/L)	863 ± 47	54 ± 29	24 ± 30	0.61
Insulin fasting (pmol/L)	13.9 ± 1.4	−2.1 ± 1.2	−1.1 ± 1.5	0.32
Insulin AUC (pmol × 120min/L)	7313 ± 815	− 464 ± 686	70 ± 723	0.24
HOMA IR	3.2 ± 0.4	**−0.7 ± 0.3**[a]	−0.3 ± 0.4	0.16
Hepatic IR	80.8 ± 12.6	**−16.9 ± 7.9**[a]	−9.9 ± 8.4	0.34
Energy consumption (kJ)	8752 ± 978	245 ± 885	1227 ± 745	0.32
Protein consumption (g)	103 ± 13.8	2.3 ± 13.1	13.7 ± 13.4	0.41
Fat consumption (g)	78 ± 11.1	0.17 ± 9.8	8.3 ± 8	0.52
Carbohydrate consumption (g)	208 ± 21.2	−0.3 ± 22.7	32.4 ± 25.5	0.27
PSA (ng/mL)	0.4 ± 0.3	0.2 ± 0.2	0.6 ± 0.3	0.33
IGF-1 (ng/mL)	132 ± 7.2	−0.6 ± 6.4	1.9 ± 6.1	0.62
IGF bioactivity (ng/mL)	0.83 ± 0.07	**0.13 ± 0.07**[a]	0.06 ± 0.05	**0.04**
IGF-2 (ng/mL)	584 ± 32.1	**−23.2 ± 12.5**[b]	1.3 ± 14.2	0.12
IGFBP-1 (ng/mL)	23.4 ± 3.4	1.5 ± 2.2	2.2 ± 2.5	0.64
IGFBP-2 (ng/mL)	210 ± 16	−8.3 ± 10.5	−15.4 ± 8.3	0.96
IGFBP-3 (ng/mL)	3722 ± 216	**−199 ± 95**[b]	92 ± 55	**0.002**
PAPP-A (ng/mL)	1.05 ± 0.1	−0.05 ± 0.04	−0.02 ± 0.02	0.13
STC2 (ng/mL)	41 ± 2.3	**−2.1 ± 1.0**[a]	−0.5 ± 1.0	0.1

Data are expressed as mean ± s.e.m; *P* values represent difference between metformin and placebo effect; bold indicates statistical significance, *P* < 0.05; [a]*P* = 0.05 compared to baseline; [b] *P* < 0.07 compared to baseline.
Cox, carbohydrate oxidation rate; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor-binding protein; LBM, lean body mass; PAPP-A, pregnancy-associated plasma protein A; PSA, prostate-specific antigen; REE, resting energy expenditure; STC2, stanniocalcin 2.

An important observation of this study is the reduction in IGF-2 during metformin treatment. IGF signalling drives cancer development and progression (22). IGF-2 shares structural similarity to insulin and in fact is a more potent mitogen than insulin when signalling through IR-A (45). IGF-2 activates the PI3K/Akt and MAPK/ERK signalling pathways (46). IGF-2 loss of imprinting, an epigenetic modification associated with aging, promotes prostate neoplastic development by increasing p-ERK signalling in a mouse model (47). In muscle cells, mTOR stimulates IGF-2 transcription and secretion (46). This is an interesting concept since metformin is a potent inhibitor of mTOR, which may therefore reduce tissue IGF-2 production. In bone marrow-derived multipotent mesenchymal stromal cells, metformin reduces IGF-2 secretion (48). Thus, metformin may inhibit IGF-2 tissue production, in line with our study showing reduction in circulating IGF-2 during metformin administration.

Previous studies have found that circulating IGF-2 is higher by about 15% in people with obesity compared to lean individuals and IGF-2 decreases following weight loss due to caloric restriction or gastric bypass surgery (49, 50, 51, 52, 53). Adipose tissue produces more IGF-2 than IGF-1, with IGF-2 being the highest in the visceral adipose tissue compared to the s.c. adipose tissue (54). This tissue-specific IGF-2 secretion may explain why weight loss may associate with reduction in IGF-2. A recent study revealed that for every mmol/L reduction in fasting plasma glucose, there was a 40 ng/mL reduction in IGF-2 following an 8-week low energy diet (52). Interestingly, there is a diet-induced reduction in IGF-2 and IGFBP-3 but not in IGF-1 (51). The expression of IGF-2 gene in blood cells is significantly higher in obese insulin-resistant compared to obese insulin-sensitive adolescents (55). Insulin has been shown to increase internalisation of IGF-2 receptors, mediating an increase in IGF-2 cellular uptake and consequent degradation (56). Thus, an improvement in insulin action/sensitivity may be linked to a reduction in IGF-2. This is in line with our study where IGF-2 had a positive association with hepatic insulin resistance at baseline and metformin treatment reduced insulin resistance with a parallel reduction in circulating IGF-2. Since IGF-2 not only exerts
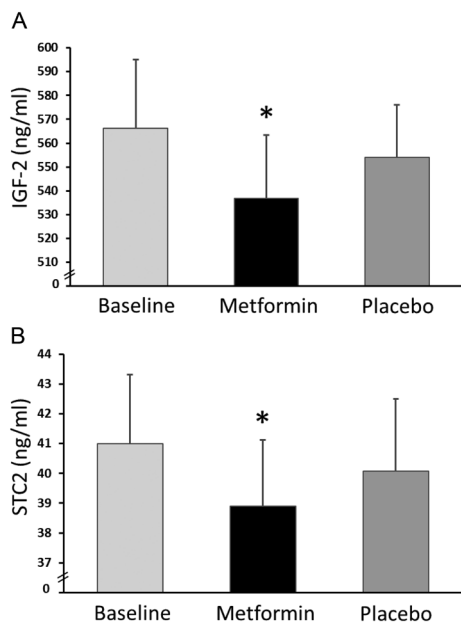
Figure 1

Serum IGF-2 (A) and STC2 (B) at baseline and during the treatment with metformin and placebo in patients with prostate cancer on stable androgen deprivation therapy. Data are expressed as means with s.e.m. * $P < 0.05$ compared to baseline. IGF-2, insulin-like growth factor 2; STC2, stanniocalcin 2.

pro-tumorigenic effects but also increases de novo androgen synthesis in prostate cancer cells (57), metformin-induced inhibition of IGF-2 provides a promising target for the anti-tumour effect of metformin.

This study also shows that during metformin treatment, there is a significant reduction in STC2, which plays a major role in cancer biology. Overexpression of STC2 is associated with cancer progression and can be used as a marker of poor prognosis (58, 59). A recent meta-analysis reports that high STC2 expression in solid cancers can serve as a tumour marker to monitor cancer development and progression (60). STC2 has oncogenic properties in prostate cancer, as overexpression promotes prostate cancer cell growth (28). Thus, STC2 could play a role in aggressive and castration-resistant prostate cancers. The mechanisms by which STC2 controls cancer growth and metastasis could be via the PI3K/AKT/Snail and AKT/ERK signalling pathways (61, 62). STC2 is also an inhibitor of PAPP-A (29), which enhances proteolytic activity of IGFBP-4 (26), upon which IGF-1 gets liberated, leading to increased IGF bioavailability (27). Thus, an increase in IGF bioactivity shown in this study may be partially explained by the reduction in STC2, potentially leaving more PAPP-A in its proteolytically active state. We also show here a significant association between the change in HOMA IR and STC2 during metformin treatment, which
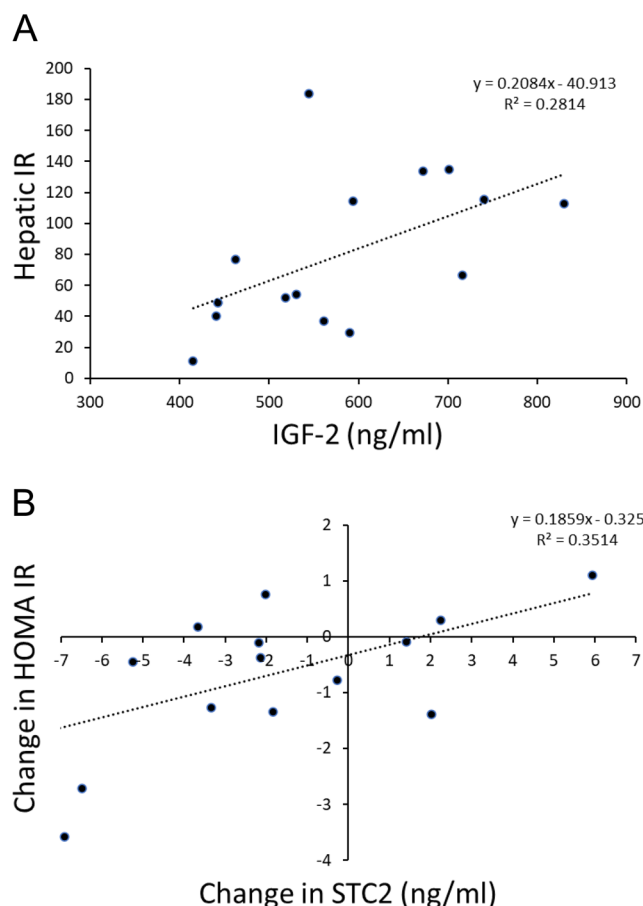

Figure 2

Associations between serum IGF-2 with hepatic insulin resistance at baseline (A) and between the change in serum STC2 and change in HOMA IR during metformin treatment (B) in patients with prostate cancer. IGF-2, insulin-like growth factor 2; STC2, stanniocalcin 2; IR, insulin resistance.

indicates that the greater the improvement in insulin sensitivity, the greater the reduction in STC2. This is supported by a recent study showing that following gastric bypass surgery, STC2 decreased and the reduction in STC2 correlated with improvements in fasting glucose, insulin, and HbA1C (53). Because STC2 can directly modify cancer growth and metastasis, the reduction in STC2 associated with metformin treatment may be another plausible mechanism for the antineoplastic effect of metformin.

IGFs circulate bound to high-affinity binding proteins, which modulate the bioavailability of IGFs. Importantly, IGFBPs have independent actions from those of IGFs on cell growth and survival (21). *In vitro* studies have demonstrated that IGFBP-3 inhibits proliferation, adhesion, invasion, and metastasis of prostate cancer, independent of IGF-1 (63, 64). IGFBP-3 is an inhibitor of MAPK signalling, which is implicated in the development of castrate-resistant prostate cancer (65). It has been also

shown that higher serum IGFBP-3 is associated with a lower risk of developing advanced-stage prostate cancer (66). Thus, higher circulating IGFBP-3 would theoretically be an advantage in prostate cancer patients, exerting direct effects on cancer cells as well as reducing IGF bioactivity. However, in this study, we show that metformin therapy reduced circulating IGFBP-3 and increased IGF bioactivity. This is an unexpected finding. However, *in vitro* studies in other cancer types have demonstrated high levels of IGFBP-3 in cancer cells, with IGFBP-3 stimulating breast cancer growth and being a poor prognostic marker for breast cancer patients (67, 68, 69, 70). Thus, it is difficult to predict how the change in circulating IGFBP-3 due to metformin treatment affects prostate cancer cells.

Research indicates that in the liver, metformin reduces GH-mediated PDK4 expression via SHP repressing hepatic gluconeogenesis (71). It is plausible that metformin may also reduce GH-mediated hepatic IGFBP-3 production. As IGFBP-3 is a principal binding protein not just for the IGF-1 but for the IGF-2 as well (72), reduction in IGF-2 seen in this study may be directly related to a reduction in IGFBP-3. Supportive of this, we neither detect a difference between the groups for the IGF-1 to IGFBP-3 ratio nor there was any significant association between the change in IGFBP-3 and the change in IGF bioactivity during metformin treatment.

An increase in IGF bioactivity may contribute to the whole-body metabolic effects of metformin. An increase in IGF bioactivity is likely to play a role in substrate metabolism and is expected to induce a muscle anabolic effect. Indeed, metformin stimulates protein synthesis in muscle (73). In adipocytes, IGF-1R activation stimulates glucose uptake. This is likely to occur also in brown fat cells, where IGF-1R activation has been shown to be essential for full thermogenic capacity (74). The increase in IGF bioactivity during metformin treatment may therefore result in a stimulation of lipid use in brown adipose tissue as well, thereby inducing increased lipid utilisation and reduction in fat mass. In line with this, we were able to demonstrate a reduction in fat mass associated with metformin treatment. An increase in IGF bioactivity is expected to improve glucose metabolism as well. Evidence comes from studies where IGF-1R deletion in skeletal muscle results in glucose intolerance and impaired insulin action and human studies reveal that IGF-1 administration enhances insulin sensitivity in patients with type 2 diabetes (75, 76, 77). Thus, the increase in IGF bioactivity during metformin administration may facilitate the beneficial effects of metformin on metabolism.

IGF-1R levels have been shown to be decreased by metformin in cell culture studies (17, 78). However, this scenario is very different from the human *in vivo* situation. Furthermore, we do not expect parallel changes in the ligand (IGF-1) and its receptor. For example, unchanged levels of ligand do not necessarily imply that the receptor IGF-1R remains unchanged or even becomes reduced (as part of a regulated response). The IGF bioactivity estimate represents the final outcome of changes in IGFBPs, PAPP-A, and STC2, as well as other proteases. Given the vast number of factors that regulate IGF bioactivity, we are limited to speculation regarding those factors that are included in this paper. Insulin is able to activate the IGF-1R, but receptor-binding experiments have shown that IGF-1R-binding affinity for IGF-2 and insulin are more than 10 and 200 times, respectively, lower than that for IGF-1. As stated, in our IGF bioactivity assay, cross-reactivity average was 0.8% for human insulin and 12% for IGF-2. Thus, insulin plays minimal role and IGF-2 a much lesser role than IGF-1 in determining IGF-1R activity. If anything, the increase in insulin sensitivity during metformin treatment would correspond to an increase in IGF-1R activity. However, it is difficult to predict what net effect would be seen in prostate cancer cells in response to an increase in circulating IGF bioactivity.

There are limitations to our study. This is a relatively small study, thus meriting confirmation of results in a larger cohort. There was no washout period between the metformin and placebo treatments. However, placebo administration was for 6 weeks before measurement of outcomes, which should have been sufficient to eliminate metformin metabolic effects. Furthermore, we undertook statistical evaluation for the order of treatment and found no effect on the results, providing evidence that there was no sequence effect in this randomised, controlled study. Nevertheless, the lack of the washout period may have reduced the apparent effect size of metformin treatment compared to placebo, if there was some carry-over of metformin action into a subsequent placebo treatment phase. We did not detect any change in PAPP-A concentrations but have to acknowledge that we did not measure its proteolytic activity, which may have increased during metformin treatment. As an increase in PAPP-A results in greater IGFBP-4 cleavage, leading to increased IGF bioactivity (26, 27), it would be of interest to measure enzymatically active PAPP-A and IGFBP-4 in this cohort of prostate cancer patients.

In summary, metformin significantly reduces circulating STC2, IGF-2, IGFBP-3 and increases IGF bioactivity. We conclude that metformin administration alters many components of the circulating IGF system, either directly or indirectly via an improved insulin sensitivity. Reduction in IGF-2 and STC2 is expected to

result in reduction in cancer growth and metastasis. This provides a possible mechanism for the proposed anticancer effect of metformin.

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