Stimulation of lactate production in human granulosa cells by metformin and potential involvement of AMP-activated protein kinase

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Précis: Metformin stimulates lactate production by human granulosa cells through a mechanism involving activation of AMP-activated protein kinase.

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Context: Production of 3-carbon units (as lactate) by granulosa cells (GC) is important in follicular and oocyte development, and may be modulated by metformin.

Objective: To examine the action of metformin on GC lactate production and potential mediation via AMP-activated protein kinase (AMPK).

Design: GC were prepared from follicular aspirates. After exposure to metformin and other potential modulators of AMPK in culture, aspects of cellular function were examined.

Setting: Private fertility clinic/ University academic centre.

Patients: Women undergoing routine *in vitro* fertilisation.

Interventions: All agents added in culture.

Main outcome measures: Lactate output of GC was measured. Cell extracts were prepared after culture and phosphorylated forms of AMPK and acetyl CoA carboxylase (ACC) assayed using Western analysis.

Results: Metformin led to a rapid increase in lactate production by GC (minimum effective dose, 250μM; maximum dose studied, 1mM (1.22-fold; $P<0.01$)). This dose range of metformin was similar to that required for stimulation of p-AMPK in GC (minimum effective dose, 250μM; maximum effect, 500μM (2.01-fold; $P<0.001$)). Increasing p-ACC, as a representative downstream target regulated by AMPK, was apparent over a lower range (minimum effective dose, 31μM; maximum effect, 250μM; $P<0.001$). A level of metformin (125μM) insufficient for the stimulation of lactate output when used alone, potentiated the effects of sub-optimal doses of insulin on lactate production. Adiponectin (2.5μg/ml) had a small but significant effect on lactate output.

Conclusions: Metformin activates AMPK in GC, stimulating lactate production and increasing p-ACC. Metformin also enhances the action of sub-optimal insulin concentrations to stimulate lactate production.

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women usually characterised by hyperandrogenism and oligomenorrhea (1). Women with PCOS often exhibit insulin resistance, which contributes to an underlying mechanism important in the pathogenesis of PCOS (2). This concept has led to the use of insulin sensitisers, such as metformin, in the treatment of PCOS.

Although the effectiveness of metformin treatment has proved variable, it is accepted that this compound can lead to improvements in symptoms associated with PCOS including a reduction in hyperandrogenism and a return to normal cycling within the ovary (3;4).

There is evidence that insulin resistance (associated with PCOS) may have an adverse effect on oocyte development and that, in *in vitro* fertilisation (IVF) cycles, this can lead to poor fertilisation of oocytes and poor developmental potential of resulting embryos (5). There is some evidence that the use of metformin *pre-conception* in IVF cycles may ameliorate these adverse effects of insulin resistance, improving clinical pregnancy rates (4). However, a clear and consistent clinical effect of metformin has been difficult to establish (6;7), with differential effectiveness of the drug reported according to the sub-group of PCOS patients studied (8).

Granulosa cells (GC) and cumulus cells (in later stages of oocyte development) are required to provide products of glycolysis for the developing oocyte, which performs this pathway only poorly (9). Although pyruvate is the key 3-carbon (3-C) intermediate transferred to the oocyte, it is likely that this is interchangeable with lactate which is the main 3-C unit produced by the GC. A lack of insulin-stimulated lactate production in GC harvested from IVF cycles of women with PCOS (10;11) may therefore point to a fundamental problem with the provision of 3-C units for oocyte development during insulin resistance.

It is established that metformin inhibits complex I of the respiratory chain thereby impairing mitochondrial function and cellular respiration (12;13). This leads to a switch to anaerobic respiration which is presumed to be responsible for elevated plasma lactate concentrations in metformin-treated patients (14), and increased levels of lactate production by tissues exposed to metformin in vitro (15). How metformin influences lactate production by GC has not been previously established. Clearly, metformin has the potential to increase lactate production by GC thereby reducing the shortfall in 3-C unit production experienced by follicular cells in insulin resistance, and improving 'nutrition' (with the supply of 3-C units) for the developing oocyte. Adiponectin, a cytokine produced by adipose cells, appears to have similar downstream effects to metformin (16), and may also, therefore, play a physiological role in maintaining nutrient supply to the oocyte.

Many of the cellular effects of metformin are mediated by AMP activated protein kinase (AMPK; (17)). AMPK is present in rat and bovine GC and appears to modulate the effects of metformin on steroidogenesis (18;19). Effective doses of metformin used in these studies (approx 1-10mM) were much higher than those required to modulate steroidogenesis in human ovarian cells (20), and also higher than circulating levels of metformin during routine clinical treatment (reported to be 10-20 μM; (21)). There is a clear need to characterize AMPK activation in human GC with respect to concentrations of metformin required for activation of the enzyme and downstream events.

The present study now addresses these issues firstly by investigating the effect of metformin on lactate production by human GC. An observed stimulatory effect is then examined in relation to the activation of AMPK, and its potential downstream effects. Implications of the study are discussed in terms of a potentially beneficial effect of metformin on 3-C unit supply to the oocyte.

Subjects and methods

Subjects

The subjects participated in the study according to a protocol which had received local ethical approval and involved a procedure of written, informed consent. The methodology involved collection of follicular aspirates obtained as part of the IVF procedure. Patients were selected with no evidence of ovarian malfunction where infertility was ascribed to either tubal blockage or problems with the male partner. Briefly, the treatment involved pituitary downregulation with a GnRH analog, induction of follicle growth with FSH, final maturation of follicles with hCG and 34h later, aspiration from follicles >15 mm in diameter (for details, see (22)).

GC preparation

Following removal of the oocytes, the aspirates (and washes) for each patient were combined and brought to the laboratory. Initially, GC were prepared according to a previously published method (22) where white blood cell contaminants were removed using antibody-bound magnetic beads, reducing white cell content to <1% of total cells. This led to a final GC suspension of approximately 10^5 cells/ml. For subsequent experiments, a simpler method of preparation, involving the removal of blood contaminants through the use of 'cell strainers', was developed as follows (providing more tissue required for Westerns and shorter-term incubations). Aspirates were first 'strained' through 40

µm 'cell strainers' (BD Biosciences, Oxford, UK), which retained cell clusters of GC. After rinsing to remove the last traces of blood contaminants, the strainers were back-washed with medium which was a mixture (50:50) of DMEM and Ham's F12 (Invitrogen, Paisley, UK), containing 10% fetal bovine serum, glutamine (2 mmol/l), penicillin (100,000 IU/l), streptomycin (100 mg/l), and amphotericin (0.25 mg/l). The resulting suspension (approx. 12 ml) was incubated at 37°C in 5% CO_2 in air for 3-4h, aspirated repeatedly through a fine 'pastette' (dispersing the clusters of GC) and then strained through a 70 µm 'cell strainer' (BD Sciences) to remove undispersed material. The suspension was diluted with about 30 ml of additional medium, centrifuged at 1200 rpm for 10 min, and the cell pellet resuspended, counted with a haemocytometer and then diluted to approx. 2-4 x 10^5 cells/ml. White cell content was estimated by a previously published method (22) and was routinely <5% of total cells. Viability, estimated by trypan blue exclusion, was routinely >95% and cells were present largely as small clusters. In general, the cells showed good attachment in subsequent culture. Omission of the 'washing' effect of the prior '3-4 h incubation/dilution/centrifugation' steps led to a variable ability of the cells to attach. Aliquots (1 ml) of cell suspension were dispensed into wells of a 24-well culture dish and cultured overnight at 37°C in 5% CO_2 in air, allowing attachment to the plastic culture surface.

Culture of GC with additions

Both methods for preparation led to a final culture in a defined medium where the serum component was substituted with transferrin (5 mg/ l), selenite (25 nmol/l) and albumin (500 mg/l; Elisa grade with low insulin concentration; Sigma, Poole, UK). Additions to cultures, formulated in defined medium, were as described in legends. 5-Aminoimidazole-4-carboximide-1-β-D-ribofuranosyl 5'-monophosphate (AICAR) and metformin were purchased from Sigma, and recombinant human adiponectin was from Peprotech (London, UK). Culture with additions continued for times indicated in legends.

Lactate assay

After culture, lactate was measured in 10 µl culture medium by a method previously described (22) which involved the use of a lactate oxidase/peroxidase reagent (Trinity Biotech, Theale, UK).

Western analysis of phosphorylated forms of AMPK and acetyl CoA carboxylase (ACC)

Immediately after culture, the culture plate with adherent cells was placed on crushed ice prior to the addition to each well of 0.25 ml of ice-cold extraction medium (100 mM Tris/HCl (pH 7.4), 10 mM sodium pyrophosphate, 2 mM EDTA, 2 mM EGTA, 100 mM sodium fluoride, 2% (v/v) protease inhibitor cocktail (Sigma No: P8340), 2% (v/v), Triton X-100, and 2 mM orthovanadate). The surface of each well was scraped with a pipette tip, the contents of each well transferred to a small tube and immediately frozen in dry ice. Each tube was then thawed, immediately homogenised using a rotating probe (Biospec Products, Bartlesville, US.), dispensed into 50 μl aliquots and rapidly re-frozen before storage at -80^{o}C.

On the day of electrophoresis, aliquots were thawed on ice before the addition of 50 μl of 2x sample buffer (125 mM Tris/HCl (pH 6.8), 4% (w/v) sodium dodecyl sulphate (SDS), 10% (w/w) glycerol, 0.006 % (w/v) bromophenol blue and 40 mM dithiothreitol). Tubes were mixed and heated for 10 min at 95^{o}C. Samples (50 μl per lane; approx. 20 μg protein) were loaded on to commercially prepared 7.5% polyacrylamide gels (Bio-Rad, Hemel Hempstead, UK) together with stained, standard molecular weight protein markers in an adjacent lane. Samples generated as part of one culture experiment were always run simultaneously and 'between-well' variation in loading was <10%. Gels were run at 90 V in buffer which was 25 mM Tris/HCl, 192 mM glycine and 0.1% (w/v) SDS. Blotting on to PVDF membrane was carried out using a semi-dry blotter (Bio-Rad) with transfer buffer which was 25 mM Tris/HCl, 192 mM glycine, 0.037% (w/v) SDS and 20 % methanol. Membranes were blocked overnight at 4^{o}C in 2% (w/v) blocker (provided as part of the ECL Advance kit, GE Healthcare, Amersham) made up in 'tris-buffered saline-tween' (TBST; 10 mM Tris/HCl (pH 7.6), 0.14 M NaCl and 1% (v/v) Tween). Blots were exposed overnight at 4^{o}C on a roller to primary antibodies dissolved in 2% (w/v) bovine serum albumin (BSA) in TBST. Primary antibodies used separately (all raised in rabbits and purchased from Millipore, Chandlers Ford, UK) were anti-phospho-AMPKα (thr172; 1:2000 dilution), anti-AMPKα2 (used at 1 μg/ml), anti-AMPKα1 (used at 1 μg/ml), and anti-phospho-ACC (Ser 79; used at 0.5 μg/ml). After washing repeatedly in TBST,

blots were then exposed for 1h at room temperature to the secondary antibody which was goat anti-rabbit IgG, HRP-conjugate (Millipore; 1:3000 dilution in TBST with 2% (w/v) BSA). After rinsing in TBST, blots were developed using the ECL Advance reagent and analysed using the Bio-Rad Versadoc system which enabled quantification of the relevant bands. Preliminary development work on GC extracts with the several anti-AMPK antibodies showed that the phosphorylated form of AMPK co-migrated (at a position equivalent to about 63kD) with a band of α_1-AMPK (confirming their identities; Fig. 2C). The α_2-AMPK isoform was not detected in the GC extracts.

Progesterone assay

This was quantified using an in-house semi-automatic immunoassay involving detection of chemiluminescence (22).

Results

Initial culture experiments on metformin and lactate production

The effect of adding metformin (500 μM) to overnight cultures of GC, purified free of white cells according to our previously described method (22), is shown in Fig. 1. A significant stimulatory effect of metformin on lactate production was observed (N=10 cultures, $P<0.002$ vs equivalent condition without metformin) irrespective of whether cells were cultured with or without additional hormones (maximal concentrations of either hCG or insulin, each of which upregulates lactate production (22)). These experiments also showed a significant inhibitory effect of metformin on hCG-stimulated progesterone production (Fig. 1, insert).

Time course studies

Having performed our initial experiments using the previously published method (22), the simpler method involving removal of blood cell contaminants by 'cell straining' was now employed providing more cellular material for Western analysis, and allowing culture experiments to be carried out over shorter time periods. Our new methodology allowed the measurement of the effect on lactate production after only 3h exposure to metformin (Fig. 2A; concn, 500μM). An equivalent time course of action on lactate production by GC was observed for AICAR (Fig 2B; concn, 4 mM), a compound known to activate AMPK. A broadly similar interval between addition of metformin and activation of

AMPK was observed (Fig. 2C and D), where the effect was becoming clearly established over 3-4.5h. Consequently, dose-response studies were standardised to a 4.5h period.

Dose-response studies

At 4.5h, media from GC cultures, previously exposed to a range of metformin concentrations, were collected and measured for lactate (Fig. 3A; N=6 experiments). Also, cells were harvested, homogenised and subjected to Western analysis for the phosphorylated α-subunit of AMPK (Fig. 3B; N=10) and phosphorylated-ACC (Fig.3C; N=5). A clear, and significant, stimulatory effect on lactate production was observed at metformin concentrations of 250μM and above (Fig 3A). The concomitant effect on AMPK activation (Fig. 3B) showed a similar overall pattern with significant stimulation at 250μM and above, although variation in the response between patients was considerable as illustrated by the error bars. The pattern of phosphorylation of ACC over the dose range of metformin (Fig. 3C) was very different with significant increases at 31μM, as the effect became established at low concentrations of the drug which were not sufficient to elicit changes in lactate. Although the '*minimum dose to elicit a significant change'* was clearly different for AMPK and ACC, it should be noted that small changes in AMPK may have occurred at doses of metformin less than 250μM but that these were masked by experimental variability.

Effect of metformin on sub-maximal insulin concentrations

With evidence that at least one downstream effect of AMPK activation was being triggered at lower concentrations of metformin, we investigated the effect of low-dose metformin on the characteristics of insulin action knowing that results would be unaffected by metformin-alone effects on lactate production. Thus, after a 4.5h exposure to 125μM metformin (a dose with no effect on lactate production when used alone) there was a significant (up to $P<0.01$) stimulatory effect of the drug on lactate production induced by sub-optimal concentrations of insulin (continued overnight) with the most significant effect seen at 3ng insulin/ml (Fig. 4).

Effect of adiponectin on lactate production

Addition of adiponectin (2.5μg/ml) to GC in culture led to a significant elevation in lactate production (Fig.5) although this was only about 10-20% of the much stronger stimulation of lactate production induced by maximal hCG (100 ng/ml). The two effects (maximal hCG and adiponectin) were not

additive (see last column). Attempts to demonstrate a significant change in p-AMPK at this adiponectin concentration gave equivocal results (data not shown).

Discussion

Metformin causes a shift towards anaerobic respiration leading to increased lactate output by tissues (14;15). Our novel data now establish that this general principle, well exemplified by work on rat muscle (15), extends to human GC which showed a marked increase in lactate production on exposure to 500 μM metformin (Fig.1). When GC lactate production was maximally upregulated with either hCG or insulin, the effect of metformin persisted suggesting that its mechanism of action is different from that of these agonists. The concomitant decrease in hCG-stimulated steroid production with metformin (insert to Fig.1) is consistent with previous work on human GC (20) and similar effects in animal species (18;19) .

Our preliminary investigations on the role of AMPK in GC confirmed its presence in the cell extracts in agreement with previous work on rat and bovine GC (18;23). The pre-dominance of the α_1-subunit (see Methods) is consistent with previous work on rat ovary (23). Time course studies with metformin and AICAR (Fig.2) revealed features of the control of lactate production by GC. Certainly, the stimulatory effect of AICAR (Fig. 2B) demonstrates that elevation of AMPK activity in GC leads to increases in lactate output presumably through effects on glucose uptake and/or glycolysis (24). This may be analogous to increases in lactate production by GC resulting from insulin-induced elevation of glucose uptake (11). Previous work has demonstrated that metformin quickly penetrates cells with end-effects on cell respiration measurable within 1h (13). AICAR has also been shown to stimulate intracellular AMPK within 1h (23). Our human GC preparation appears to have an extra time delay built into the system with onset of lactate responses to metformin and AICAR (Figs 2A and B), and activation of AMPK with metformin (Fig. 2C and D) all measurable after about 3h.

Explaining metformin dose-response relationships can be problematic as levels used for *in vitro* work are often high compared with normal levels of exposure during clinical treatment. Certainly, treatment *in vivo* (where the circulating concentrations are 10-20μM) may be accompanied by slow accumulation within tissues (21). This may partially explain why higher levels are needed in short

term incubations *in vitro* where time is not available for accumulation. In the present study, effective levels of metformin with regard to lactate production (Fig 3A) and AMPK activation (Fig. 3B) were 250μM and above, and were broadly in line with concentrations used in previous studies on rat GC (e.g., (18)). The close parallel between these two parameters is consistent with a causative link between p-AMPK and lactate output. However, much lower concentrations of metformin (approx. 30μM and above) were effective with regard to ACC phosphorylation (Fig. 3c) suggesting that downstream effects of AMPK may be manifest at concentrations of metformin below the threshold for lactate production. The explanation for this is obscure but may be to do with the considerable between-patient variability in levels of p-AMPK at lower doses of metformin, which could hide small but important changes in activity. We speculate that there is magnification built into the AMPK cascade whereby small changes in p-AMPK lead to larger changes in downstream events. Our observations may offer a partial explanation for the report by Mansfield *et al.* (20) which described effective doses of metformin in the sub-micromolar range, although further allowance would be needed for accumulation of the drug in the extended culture of 48h used in that study.

It is established that AMPK has a wide range of targets within cells so that activation has the combined effect of increasing energy supply and decreasing energy consumption in macromolecular synthesis (24). We argue that the lower concentration range of metformin required for increasing p-ACC in GC may provide a guide to the levels of the drug required for this wider range of target alterations. It has been suggested that one such target lies within a mechanism governing insulin sensitivity such that metformin is able to increase sensitivity to insulin with regard to glucose disposal in muscle (reviewed by (25)). Our finding shown in Fig. 4 that the effect on GC of sub-optimal doses of insulin appears to be potentiated by metformin at a concentration (125 μM) unable to stimulate lactate production when used alone (Fig. 3A), shows that this mechanism may apply in GC (like skeletal muscle) and provides extra evidence for our assertion that p-ACC provides a guide to the alterations of other AMPK targets despite being associated with minimal overall changes in p-AMPK at lower concentrations of metformin.

The interaction of metformin and insulin action on GC is emerging as a mixed picture. On the one hand, it is clear that metformin has the potential to reinforce the action of sub-optimal doses of insulin on lactate production (probably through an effect on glucose uptake). On the other, an inhibition of fatty acid synthesis through increasing p-ACC would tend to offset any positive effect of insulin on fatty acid synthesis through increasing SREBP-1c as previously observed in this laboratory (19).

There are parallels between the modes of action of metformin and the naturally occurring adipocytokine, adiponectin, as both appear to operate through stimulation of AMPK to enhance insulin action (16). It was difficult to arrive at an appropriate level of adiponectin to include in cultures of GC as adiponectin circulates over a considerable concentration range in humans (3-30 µg/ml) and is present in a number of molecular forms (26). Using 2.5µg/ml we nevertheless observed a significant effect on lactate output (Fig. 5) though the effect was lower than the strong effect of hCG expected in this system (22). The observation that the effects of the two agents, adiponectin and hCG, were not additive suggests that their modes of action are different. Our results are consistent with the observed presence of adiponectin receptors in rat ovarian tissue (27) and human GC (unpublished work of this laboratory), and point to a physiological role for adiponectin in ovarian function. Perhaps our work on metformin acts as a model system for how adiponectin might facilitate insulin action in the ovary.

It is difficult to extrapolate from in vitro findings to the situation in vivo, where 3-C unit production for the oocyte will be influenced by a complex range of factors including insulin, gonadotrophins, oocyte maturation and follicle size (28-30). For example, measurements of glucose and lactate in follicular fluids obtained from PCOS patients with increased insulin resistance (based on average figures for the group vs controls) do not appear to show a disordered relationship between these two metabolites in PCOS (31). Also, measurements of follicular components in obese, normoandrogenic women with raised circulating and follicular insulin, failed to show concomitant changes in follicular glucose and lactate (32). These in vivo studies contrast with two independent studies showing lack of insulin-induced lactate production in vitro by GC from follicles of women with PCOS (10;11).

Recognising these difficulties we put forward the following schema (shown in Fig. 6), based on the in vitro evidence, which attempts to put our work into context. The oocyte performs glycolysis only poorly and depends on the provision of 3-C units through the agency of the surrounding cumulus cells. Because cumulus cells appear to have the ability to use imported lactate to make pyruvate (discussed by (9)), we suggest that lactate generally within the follicle (mostly made by the GC; (30)) may form part of a co-ordinated system for transferring 3-C units to the oocyte. The metabolism of GC within follicles appears to be almost entirely anaerobic with 2 molecules of lactate being produced from each molecule of glucose taken in (28). Consequently, the drive to supply 3-C 'nutrient' to the oocyte must be very dependent on insulin-enabled glucose uptake by follicular cells. In insulin resistance, the transfer of 3-C units to the oocyte is compromised (10;11) and this can lead to problems with oocyte development . Our work, now shows that up-regulation of AMPK with metformin (or through the physiological impact of adiponectin) could potentially increase lactate production oncemore (Fig.6). It should be stressed that the present study has not used cells from patients with PCOS and therefore has not tested directly the central idea shown in figure 6. However, our work suggests the need for further studies on GC specifically linked to data on insulin resistance of the donor patients. In this way, the impact of whole-body insulin resistance on GC could be determined and the potential for the reversal of deficiencies with metformin established. Despite the limitations of our study, it provides a potential and testable rationale for the observed effects of metformin therapy (4) in the treatment of PCOS.

In summary we have shown that metformin acts on human GC by activating AMPK and that this is associated with an increase in lactate output. A number of downstream targets of AMPK are altered including ACC and a mechanism governing responses to sub-optimal levels of insulin.

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FIG. 1. Effect of metformin (500μM) on lactate production by human GC. Cultures were for 16h without additional hormones (control), with maximal hCG (100 ng/ml), or with maximal insulin (1000ng/ml), and were either with metformin (hatched bars) or without (open bars). Lactate output is expressed as percent of control values (without metformin). Insert shows equivalent values for progesterone expressed as nmol/l. Bars represent means (with SEM; n=10 experiments for lactate and n=4 for progesterone). *, P<0.05; **, P<0.002 vs. equivalent condition without metformin (paired t-test).

FIG. 2. Short-term time course studies on the effect of metformin (500μM) and AICAR (4mM) on lactate output by GC, compared with the onset of metformin-induced activation of AMPK. A), Effect of metformin (dashed line) on lactate vs control (solid line). B), Effect of AICAR (dashed line) on lactate vs control (solid line). Error bars (some too small to be visible) show SEMs reflecting variation within the typical experiment shown. C), Western blot showing α_1-subunit (α_1-) of AMPK in non-incubated cells (left-hand panel) and blot showing p-AMPK (right-hand panel) increasing after 500μM metformin treatment and D), values for p-AMPK, calculated through image analysis of the bands visible on blot C, for metformin treatment (dashed line) vs control (solid line). `.

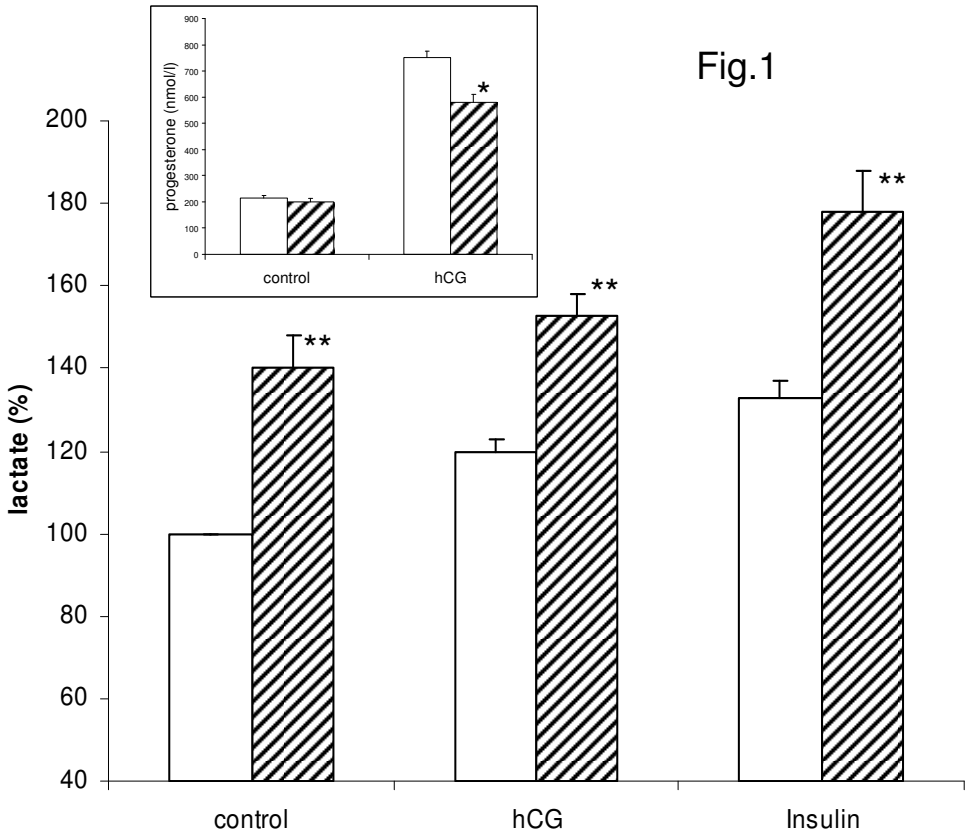
FIG. 3. Dose-response relationships for metformin action on GC with regard to lactate output (A), AMPK activity as reflected by p-AMPK (B), and phosphorylation of ACC (D) as an accepted, representative downstream target for AMPK. Lactate is expressed as percent of control (no metformin) whereas p-AMPK and p-ACC are as percent of maximum response measured in each experiment. Values represent means (with SEM; n=6 experiments for lactate, n=10 for p-AMPK and n=5 for p-ACC). *, P<0.05; **, P<0.01; ***, P<0.001 vs control (paired t-test).

FIG. 4. Effect 125μM metformin (insufficient to cause lactate increase when used alone) on GC lactate output in the presence of a range of concentrations of insulin. The GC were incubated with metformin for 4.5h before addition of the insulin and then cultured for 16h with the insulin and metformin where appropriate. Values represent means (with SEM; N=6). P values shown and calculated as in previous legend.

FIG. 5. A comparison of the effects of adiponectin (2.5μg/ml), maximal hCG (100ng/ml), and both agonists together on lactate production by GC. Bars represent means (with SEM; n=6 experiments). *, P<0.05 vs control (paired t-test).

FIG. 6. Delivery of 3-C units to the oocyte. We envisage insulin-dependent glucose (G) uptake by both GC and cumulus cells. Through respiration which is predominantly anaerobic, G is converted to pyruvate (P) which is interchangeable with lactate (L) through the agency of lactic dehydrogenase. Large quantities of L subsequently released into the follicular fluid should be available to the cumulus cells for conversion back to P and for transfer to the developing oocyte, augmenting the intrinsic capacity of cumulus cells to produce P. Under normal conditions of insulin sensitivity, there is efficient delivery of 3-C units to the oocyte. In insulin resistance, the action of insulin to sustain glucose uptake is reduced so that efficient production of 3-C units is compromised. Clearly, activation by metformin or adiponectin of AMPK has the potential to increase oncemore the delivery of 3-C units to the oocyte mitigating the effects of insulin resistance.

Fig.1


lactate (%)
200
180
160
140
120
100
80
60
40
control
hCG
Insulin
**
**
**
progesterone (nmol/l)
900
800
700
600
500
400
300
200
100
0
*
control
hCG

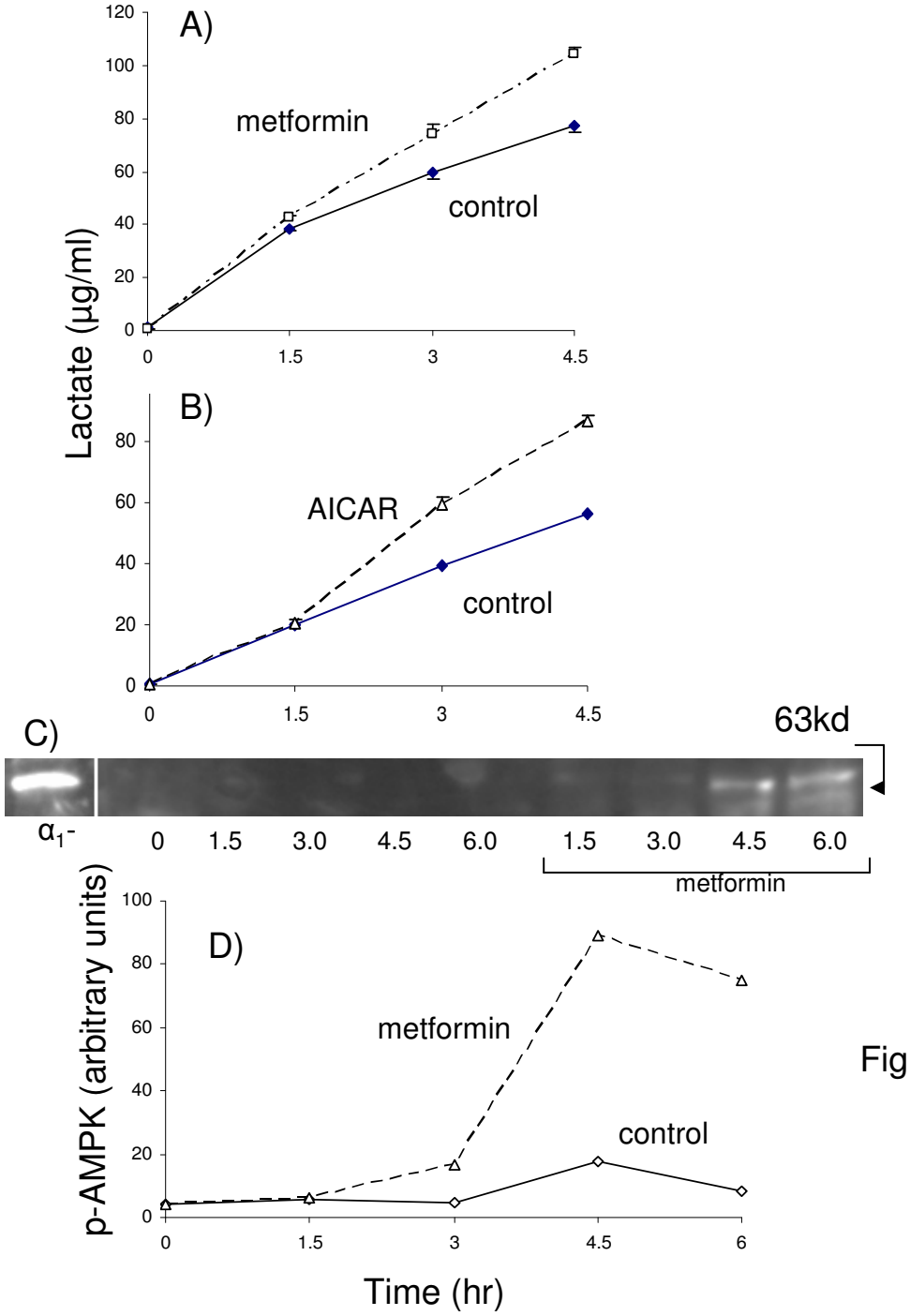

A)
metformin
control
Lactate (µg/ml)
B)
AICAR
control
C)
63kd
α1-
0 1.5 3.0 4.5 6.0 1.5 3.0 4.5 6.0
metformin
D)
metformin
control
p-AMPK (arbitrary units)
Time (hr)

Fig 2

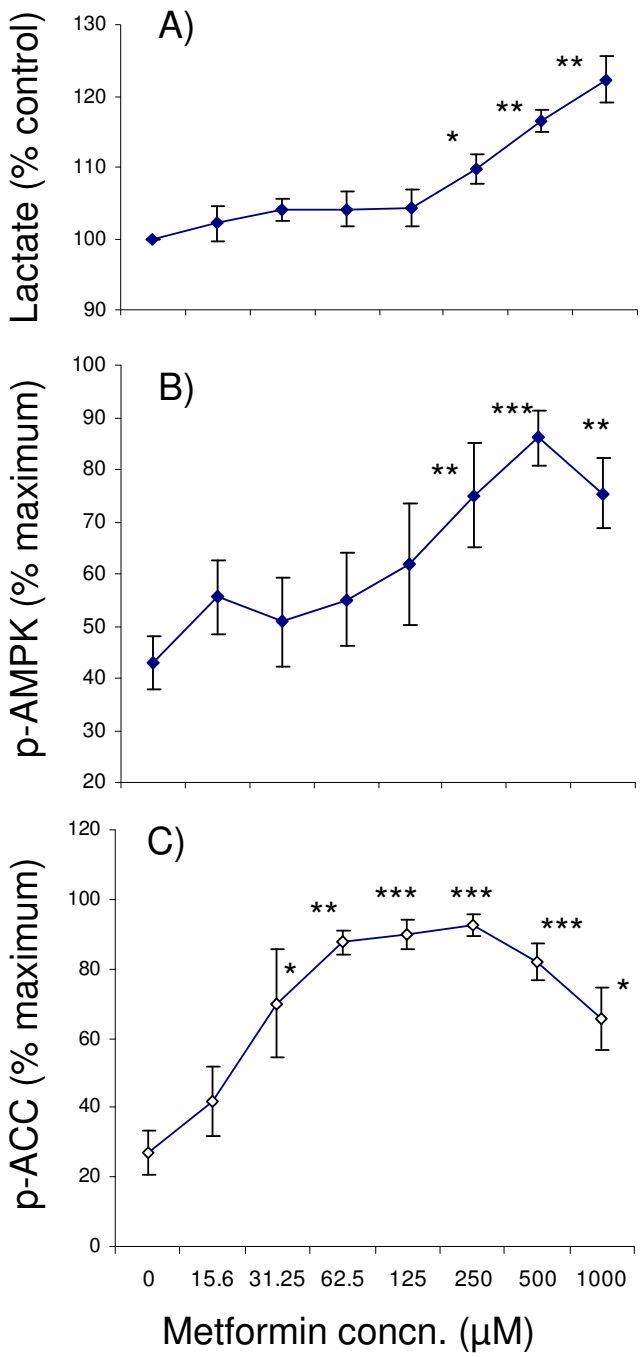

A)
Lactate (% control)
130
120
110
100
90
*
**
**
B)
p-AMPK (% maximum)
100
90
80
70
60
50
40
30
20
**
***
**
C)
p-ACC (% maximum)
120
100
80
60
40
20
0
*
**
***
***
***
*
0
15.6
31.25
62.5
125
250
500
1000
Metformin concn. (µM)

Fig 3

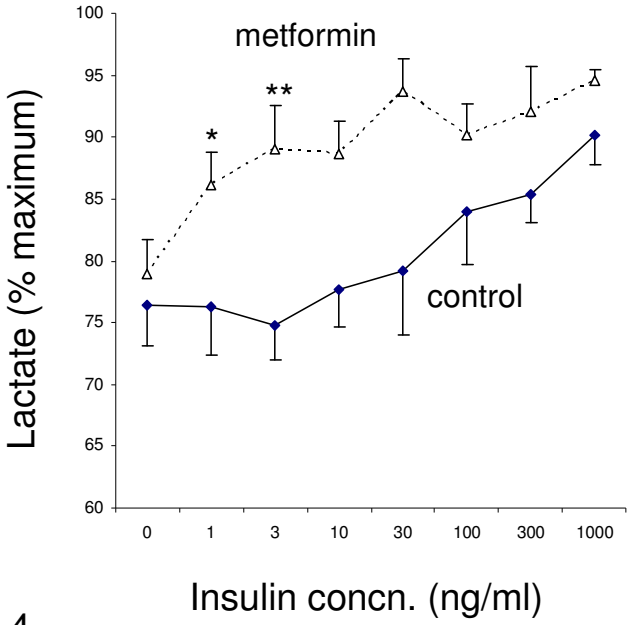

metformin
control
*
**
Lactate (% maximum)
Insulin concn. (ng/ml)
100
95
90
85
80
75
70
65
60
0
1
3
10
30
100
300
1000

Fig 4

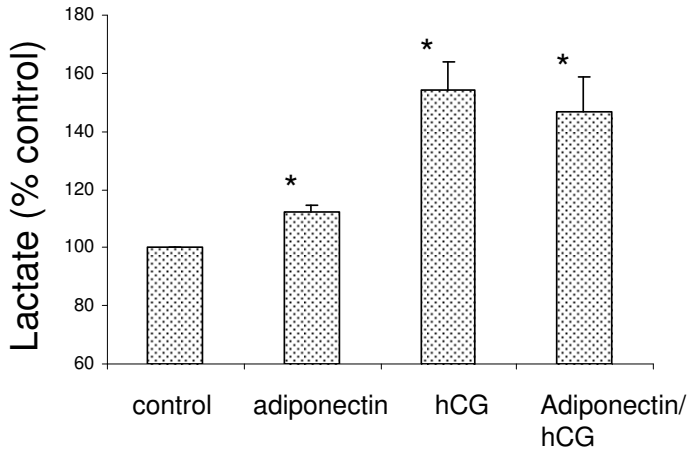
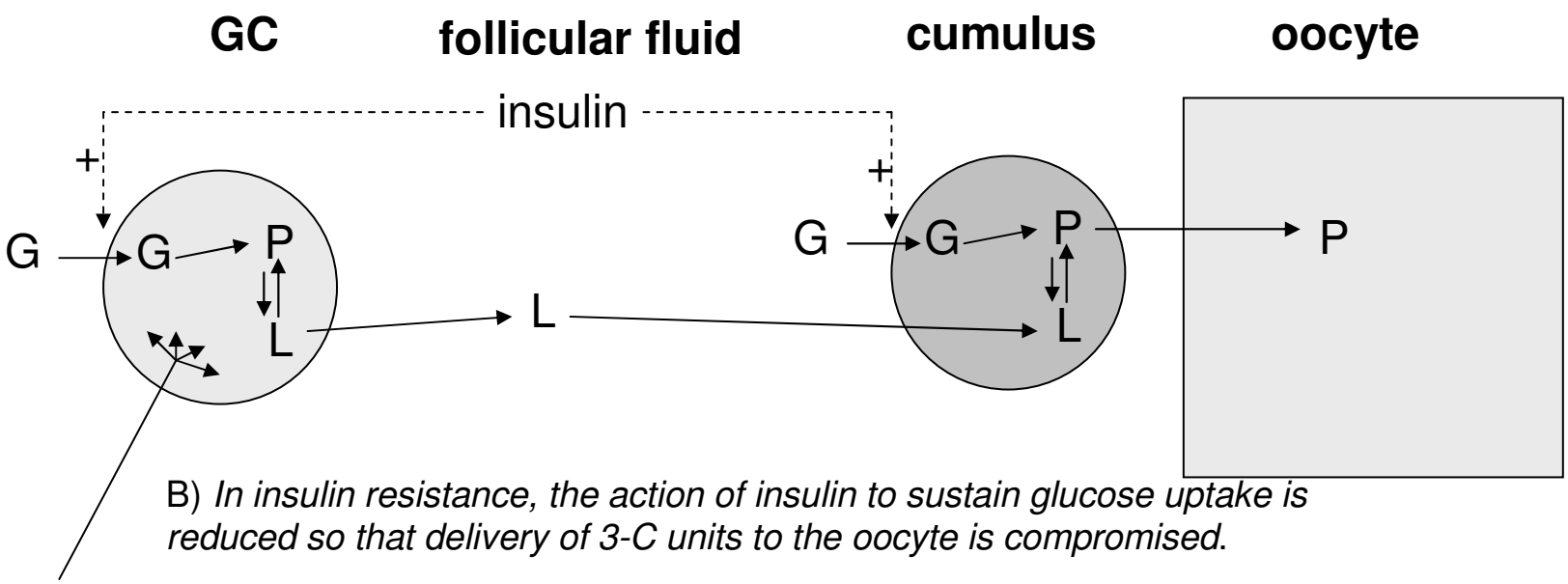

Lactate (% control)
180
160
140
120
100
80
60
*
*
*
control
adiponectin
hCG
Adiponectin/
hCG

Fig.5

A) *Normal physiological situation giving efficient drive of 3-C unit delivery to oocyte*:



B) *In insulin resistance, the action of insulin to sustain glucose uptake is reduced so that delivery of 3-C units to the oocyte is compromised*.

C) *AMPK activators (metformin/adiponectin) can restore delivery*.

Fig 6