# DYDROGESTERONE AND NORETHISTERONE REGULATE EXPRESSION OF LIPOPROTEIN LIPASE AND HORMONE-SENSITIVE LIPASE IN HUMAN SUBCUTANEOUS ABDOMINAL ADIPOCYTES

SL Palin, Division of Medical Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TH.

PG M$^{c}$Ternan, University of Warwick, Clinical Sciences Research Institute, Clifford Bridge Road, Coventry, CV2 2DX.

KC McGee, University of Warwick, Clinical Sciences Research Institute, Clifford Bridge Road, Coventry, CV2 2DX.

DW Sturdee, Dept of Women's Health, Solihull Hospital, Lode Lane, Solihull B91 2JL.

AH Barnett, Division of Medical Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TH.

S Kumar, University of Warwick, Clinical Sciences Research Institute, Clifford Bridge Road, Coventry, CV2 2DX.

Corresponding Author:

Dr SL Palin, Department of Medicine, Division of Medical Sciences, Queen Elizabeth Hospital, University of Birmingham, Edgbaston, Birmingham B15 2TH.

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Tel: 07976 311885

Fax: 0121 6272384

Email: s.l.palin@bham.ac.uk

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**Abstract**

**Aim:** In premenopausal women, hyper-androgenicity is associated with central obesity and an increased cardiovascular risk. We investigated the effects of dydrogesterone (DYD)(a non-androgenic progestogen) and norethisterone (NET)(an androgenic progestogen) on lipoprotein lipase (LPL), hormone-sensitive lipase (HSL) and glycerol release in adipocytes isolated from subcutaneous abdominal adipose tissue. **Methods:** Adipose tissue was obtained from 12 non-diabetic women, mean age 51 years (range 37-78) and mean BMI 25.4kg/$m^2$ (range 20.3-26.4). Adipocytes were treated with increasing doses of DYD and NET for 48 hours prior to protein extraction. Effects on lipogenesis and lipolysis were assessed using western blotting to determine the expression of key enzymes, LPL (56kDa) and HSL (84kDa) respectively. Measurement of glycerol release into the medium provided an assessment of lipolytic activity. **Results:** Expression of LPL was increased by DYD and NET (mean protein expression relative to control ± SEM); with greatest effect at $10^{-8}$M for DYD: 2.32±0.51($p<0.01$) and $10^{-8}$M for NET: 2.06±0.19($p<0.01$). In contrast, HSL expression was reduced by all concentrations of DYD, with maximal effect at $10^{-9}$M: 0.49±0.02($p<0.001$). NET reduced HSL expression at all concentrations from $10^{-9}$M: 0.62±0.06($p<0.001$) to $10^{-7}$M: 0.69±0.08($p<0.001$). Glycerol measurements supported the HSL expression studies($p>0.05$). **Conclusions:** DYD and NET significantly increased LPL expression relative to control whilst significantly reducing HSL expression. At the concentrations studied, similar effects were observed with the androgenic NET and the non-androgenic DYD despite differing effects on the lipid profile when taken

in combination with estrogen. Further work in this area may improve knowledge about the effects of different progestogens on body fat distribution and enable progestogen use to be tailored to the individual to achieve maximal benefits.

## Introduction

Oral estrogen therapy (ET) is associated with a beneficial reduction in total and low density (LDL)-cholesterol and an increase in high density (HDL)-cholesterol, but also a potentially deleterious increase in serum triglycerides suggesting increased lipolytic activity [1-5]. Recent clinical studies suggest that ET may have beneficial effects on the central body fat [6] that is recognised to accumulate following the menopause [7] and which is associated with increased cardiovascular risk [8]. Similarly, clinical studies in men associate testosterone deficiency with central obesity [9,10], with testosterone appearing to reverse this adiposity suggesting that androgen deficiency in men is associated with central obesity [11]. In premenopausal women, hyper-androgenicity is associated with central obesity [12], which raises concern about the possible androgenic effects of the progestogen component of combined estrogen and progestogen therapy (EPT).

Progestogen is required to protect against uterine carcinoma observed in women with an intact uterus using unopposed estrogen [13]. Progestogens may reduce the beneficial effects of estrogen on the lipid profile but may also be beneficial [14]. The 19-nortestosterone derivatives e.g. norethisterone (NET) possess androgenic properties, whilst the C21 progestogens e.g. dydrogesterone (DYD) are less androgenic [15]. In a pooled analysis of oral EPT, DYD did not oppose the adverse rise in triglycerides or the beneficial rise in HDL-cholesterol seen with ET. NET induced a net reduction in triglycerides but also a net reduction in HDL-cholesterol [14]**.** Clinical studies examining the effects of EPT on body fat distribution have also suggested beneficial effects [6, 16-18].

Fat mass is controlled by the processes of lipogenesis and lipolysis, with the key regulatory enzymes being lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) respectively. Most animal and human studies of progestogens, in the presence of

estrogen, have shown increased adipose tissue LPL activity [19-25], although not all progestogens used in EPT have been studied. There is less data on the effects of progestogens on lipolysis with no clear overall picture having emerged [21, 24-26]. Our aim was to investigate effects of a non-androgenic progestogen, DYD, and an androgenic progestogen, NET, on lipogenesis and lipolysis in subcutaneous abdominal adipocytes isolated from women through measurement of LPL and HSL respectively. In addition, we investigated the rate of lipolysis through measurement of the glycerol concentration in the media.

## Materials and Methods

### Isolation of mature adipocytes

Subcutaneous abdominal adipose tissue was obtained from 12 non-diabetic subjects (mean age 51 years (range 37-78), mean BMI 24.5 $kg/m^2$ (20.3-26.4). Samples were obtained during elective surgery in accordance with guidelines from South Birmingham ethics committee. Women using glucocorticoids, levothyroxine, oral contraceptives, ET, EPT and lipid-lowering therapies were excluded.

Adipocytes were isolated using previously described methods [27, 28] and cultured in phenol red-free Dulbecco's modified Eagle's medium (DMEM/F12)(Gibco, UK) with penicillin (100U/ml), streptomycin (100μ/ml) and transferrin (5μ/ml)(Sigma, UK). Compacted 1ml aliquots of adipocytes (approximately 500,000 cells) were treated with DYD (Solvay Pharmaceuticals, UK) and NET (Sigma, UK) from $10^{-9}$M to $10^{-7}$M and maintained for 48 hours. Samples without hormones added were maintained as controls. Due to the large number of treatments involved, it was not always possible to obtain sufficient material for all of the treatments to be studies in each patient. As previously

described, assessment of adipocyte viability, extraction and quantification of protein was performed [27, 28].

**Analysis of samples**

Equal amounts of protein for each treatment and control (10-50μg) were analysed using Western blotting as previously described [27, 28]. Primary antibodies against LPL (56kDa)(1 in 5000 with BSA 0.05%; Research Diagnostics Inc, USA) or HSL (84kDa)(1 in 500) [29] were used, with anti-mouse and anti-rabbit secondary antibody respectively (Binding Site, UK). Chemiluminescent detection was then utilised (ECL+ for LPL and ECL for HSL; Amersham Pharmacia Biotech, UK), with quantification of radiographs using Windows: Gelbase/Gelblot (UVP Ltd, UK).

The glycerol concentration in the media samples were analysed in triplicate using a commercially available colourimetric method (Randox Laboratories, UK).

Statistical analysis was undertaken using analysis of variance for the comparison of control against treatment samples.

**Results**

**Effects on LPL protein expression**

All concentrations of DYD increased LPL protein expression, with the maximal effect observed at $10^{-8}$M; mean protein expression relative to control ± standard error; DYD$10^{-8}$M: 2.32±0.51($p<0.01$)(Figure 1). Similarly, all concentrations of NET increased LPL protein expression, with the maximal effect at NET$10^{-8}$M: 2.06±0.19 ($p<0.01$)(Figure 1).

**Effects on HSL protein expression**

DYD reduced expression of HSL protein with increasing concentrations, with the maximal effect observed at DYD$10^{-9}$M: 0.49±0.02($p<0.001$)(Figure 2). Similarly, HSL

protein expression was reduced by NET at all concentrations from NET$10^{-9}$M: 0.62±0.06($p<0.001$) to NET$10^{-7}$M: 0.69±0.08($p<0.001$)(Figure 2).

**Glycerol release studies**

Lower glycerol concentrations were detected in the medium compared to control from $10^{-9}$M to $10^{-7}$M for both DYD and NET ($p>0.05$) (data not shown). These data are in agreement with the HSL protein expression studies.

## Discussion

We present the first *in vitro* evidence that DYD and NET have regulatory effects on lipogenesis and lipolysis in subcutaneous abdominal adipocytes isolated from women. Addition of DYD and NET increased LPL protein expression and reduced HSL protein expression, suggesting increased lipogenesis and reduced lipolysis respectively. These results confirm that progestogens may regulate adipose tissue mass through effects on the net amount of adipose tissue in the adipocyte. Together with evidence that progesterone increases adipocyte number through effects on proliferation and differentiation in rats [30, 31], our findings suggest that progesterone has a role in the regulation of fat mass. Despite evidence that HT (hormone therapy) does not cause weight gain, many women express concern about gain weight when considering HT [32]. Further information about effects of EPT on body fat may help alleviate some of the fears that women have about weight gain.

The effects of the progestogens on glycerol release, whilst not statistically significant, suggested a trend consistent with the HSL expression studies. The absence of statistical significance might be due to increased cell lysis leading to increased glycerol release and greater variability in the samples.

The protein expression studies are in keeping with studies of adipose tissue in intact female rats showing that progesterone alone [19–21], and in combination with estrogen, increased LPL activity [19,22]. Few studies have examined the effects of progesterone on lipolysis in rats although no effect was seen in intact female rats [21] or ovariectomised and adrenalectomised rats [25]. Further work using progesterone both alone and with estrogen, in ovariectomised and adrenalectomised rats [25, 33-35], concluded that estrogen is required for the effects of progesterone on lipogenesis and lipolysis to be seen [25]. In our study, estrogen was not added to the isolated adipocytes since the aim was to study the effects of the progestogens alone. The adipocytes had, however, been exposed to estrogen prior to isolation since the cells were obtained from women and even postmenopausal women are recognised to possess measurable plasma and adipose tissue levels of estrogen [36].

Previous *in vivo* human work showed that percutaneous progesterone applied to the thigh for 24 hours, during the follicular phase of the menstrual cycle, increased LPL activity in femoral adipose tissue [23] in agreement with our findings. Oral 17β-estradiol ($E_2$), in combination with androgenic progestogens; levonorgestrel or sequential medroxyprogesterone acetate, was found to increase LPL activity significantly in subcutaneous adipocytes from the femoral area, but not to have significant effects in the abdominal area [24]. These results suggest a modulating role for progestogens, since alone $E_2$ reduces lipogenesis in adipocytes from subcutaneous abdominal [28] and gluteal adipose tissue [39,40]. The combination of $E_2$ and levonorgestrel did not significantly alter lipolysis measured by glycerol release [24] suggesting that the progestogen may also modulate the effects of $E_2$ on lipolysis since $E_2$ alone has been shown to increase lipolysis in animal [24, 41-42] and human studies [28]. Work using oral ethinyl estradiol (EE) alone, and in combination with NET, gave

differing results [26] that might be due to the doses of estrogen used or the different systemic potencies of $E_2$ and EE [43].

In summary, in subcutaneous abdominal adipocytes isolated from women, the progestogens increased LPL protein expression and reduced HSL protein expression relative to control. Similar effects were observed with androgenic NET and non-androgenic DYD at the concentrations studied despite the differing effects on the lipid profile when each are taken with estrogen as oral EPT [14]. The concentration of progestogen in fat following oral administration is not known, however, and may differ dramatically to the concentrations used and could also differ with the progestogen. Additional information might be obtained through similar studies using lower hormone concentrations in this adipose tissue depot and also in other adipose tissue depots. The discovery in human adipose tissue of the estrogen receptor (ER) [44], the progesterone receptor (PR) and its isoforms (PR-A and PR-B) [45], provides further opportunities to determine how sex steroids influence body fat distribution. Consideration is increasingly been given to the progestogen chosen for combination with estrogen in HT. Additional work in this area may enable the progestogen in EPT to be further tailored to the individual woman to optimise effects on both the lipid profile and body fat distribution from the perspective of cardiovascular risk.

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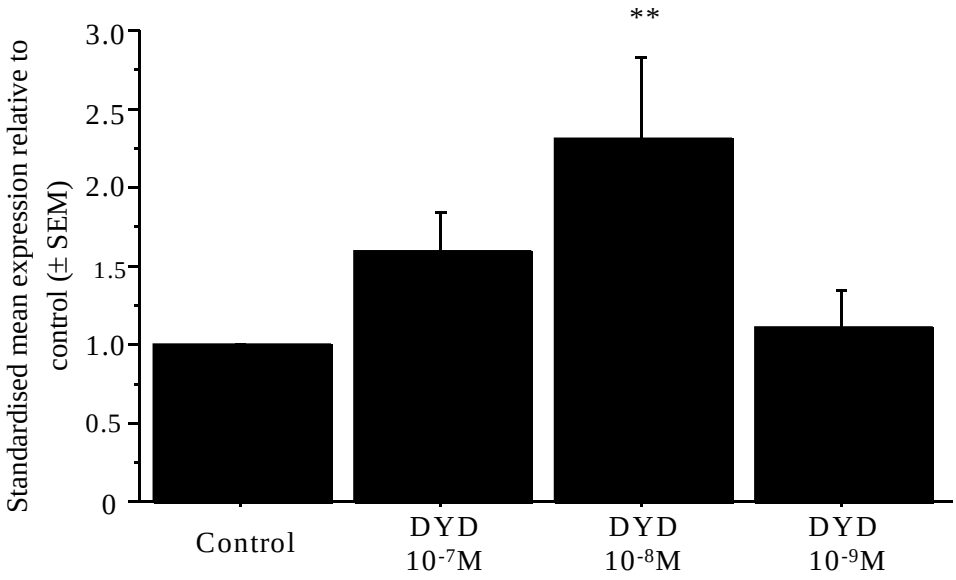
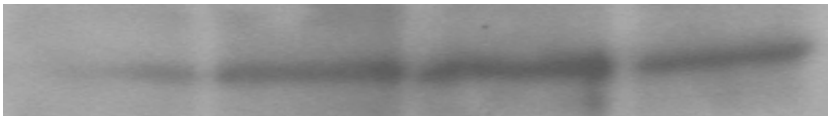
**Figure 1**

**A**


Standardised mean expression relative to control (± SEM)
3.0
2.5
2.0
1.5
1.0
0.5
0
**
Control
DYD 10-7M
DYD 10-8M
DYD 10-9M

**B**



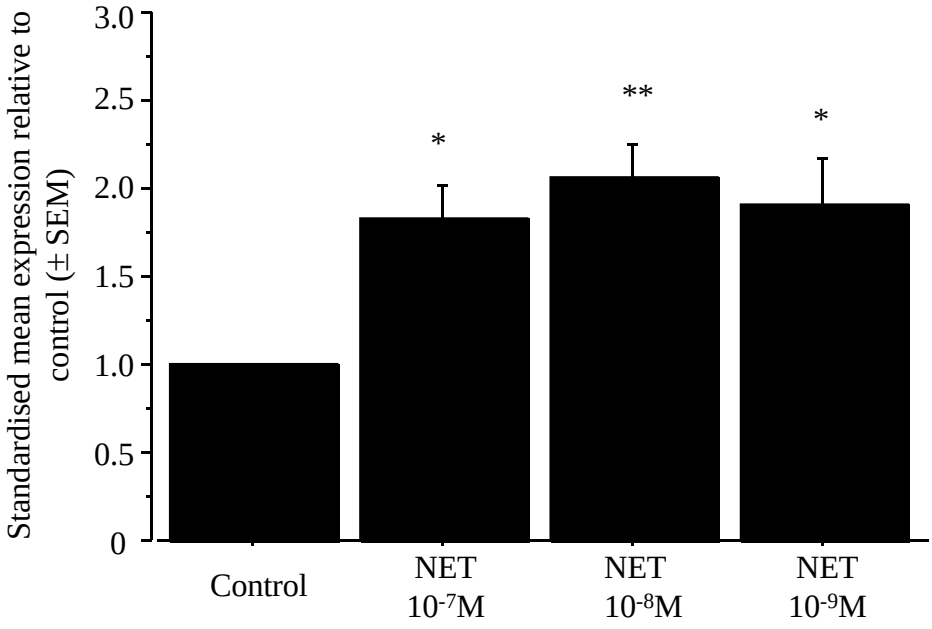
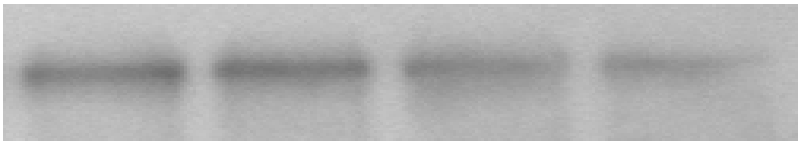
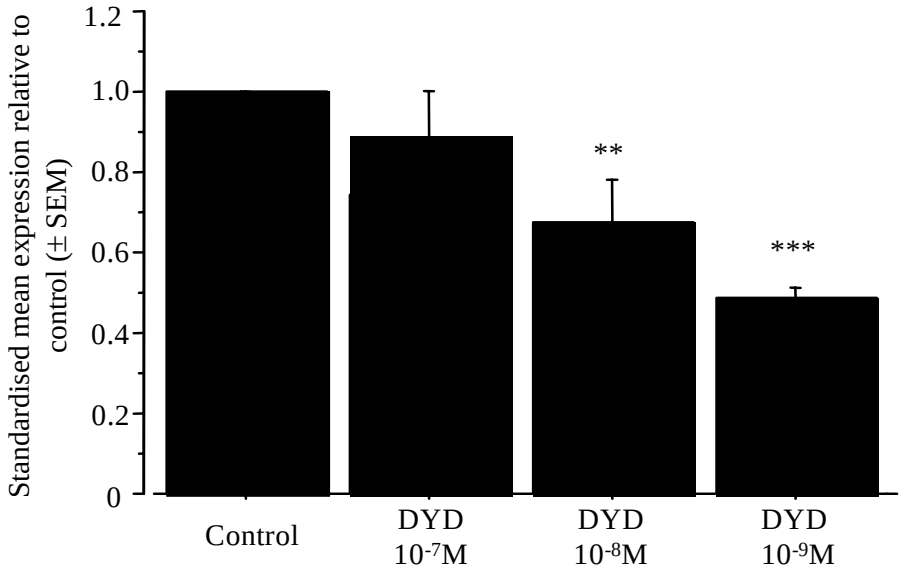

Standardised mean expression relative to control (± SEM)
3.0
2.5
2.0
1.5
1.0
0.5
0
*
**
*
Control
NET 10-7M
NET 10-8M
NET 10-9M

**Figure 2**

**A**




Standardised mean expression relative to control (± SEM)
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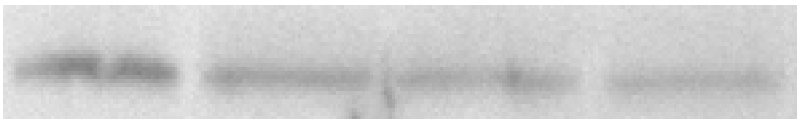
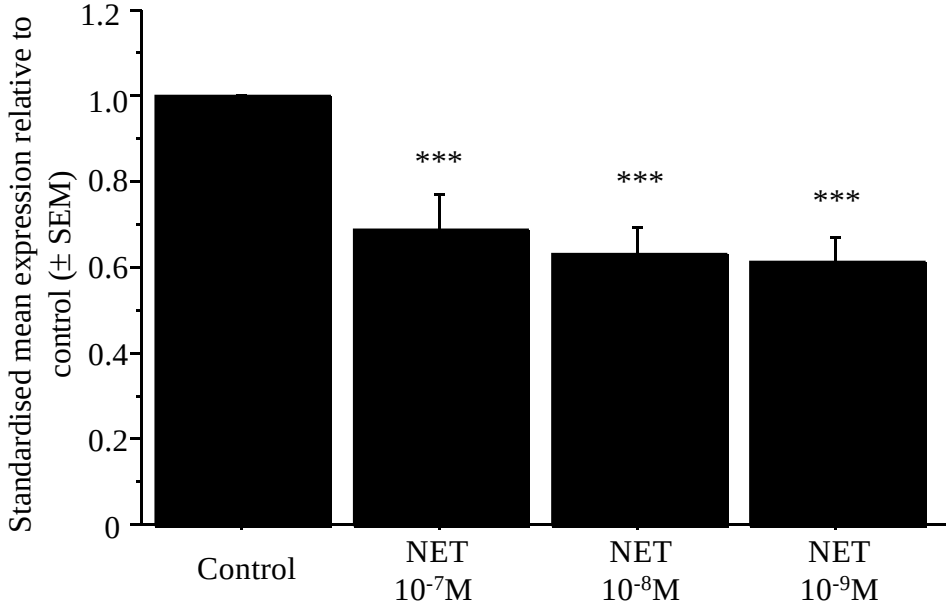
1.0
0.8
0.6
0.4
0.2
0
**
***
Control
DYD 10-7M
DYD 10-8M
DYD 10-9M

**B**




Standardised mean expression relative to control (± SEM)
1.2
1.0
0.8
0.6
0.4
0.2
0
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Control
NET 10-7M
NET 10-8M
NET 10-9M

**Figure Legends**

**Figure 1.** These graphs show the mean protein expression relative to control for (A) dydrogesterone (DYD)($10^{-9}$M to $10^{-7}$M) (n=4) and (B) norethisterone (NET)($10^{-9}$M to $10^{-7}$M) (n=4) for lipoprotein lipase in isolated subcutaneous abdominal adipocytes from women. Values expressed as mean ± standard error, with a representative western blot shown for both graphs and p values; *p<0.05, **p<0.01.

**Figure 2.** These graphs show the mean protein expression relative to control for (A) dydrogesterone (DYD)($10^{-9}$M to $10^{-7}$M) (n=6) and (B) norethisterone (NET)($10^{-9}$M to $10^{-7}$M) (n=4) for hormone-sensitive lipase in isolated subcutaneous abdominal adipocytes from women. Values expressed as mean ± standard error, with a representative western blot shown for both graphs and p values; *p<0.05, **p<0.01, ***p<0.001.