**Supplemental Table 1.** Study variables in control girls (N= 31) and in girls with polycystic ovary syndrome. Girls with PCOS were randomized to receive oral contraception (OC, Ethinylestratiol 20 µg plus Levonorgestrel 100 mg) or a low-dose combination of Spironolactone (50mg/ d), Pioglitazone (7.5 mg/ d) and Metformin (850 mg/ d) (SPIOMET) for 1 year. Baseline data are presented for N= 30 girls with PCOS. Longitudinal data are shown from N= 23 girls with PCOS (N= 12 on OC; N= 11 on SPIOMET) with available stool samples for microbiota analysis at baseline and after 1 year.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | **Controls** | **PCOS** | **OC (N= 12)** | | | | | | | **SPIOMET (N= 11)** | | | |
|  | | **(N= 31)** | **(N= 30)** | **Start a** | | **1 year** | | | ***Δ 0-1 year*** | | **Start a** | **1 year** | ***Δ 0-1 year*** | |
| **Auxology** | | | | | | | | | | | | | | |
| Age (years) | | 15.9 ± 0.2 | 15.8 ± 0.3 | 15.6 ± 0.5 | | - | | | *-* | | 15.6 ± 0.4 | - | *-* | |
| Birth weight Z-score | | 0.1 ± 0.2 | -0.6 ± 0.2 \*\* | -0.8 ± 0.3 | | - | | | *-* | | -0.5 ± 0.3 | - | *-* | |
| BMI (kg/ m2) | | 22 ± 0 | 25 ± 1 \*\* | 25 ± 1 | | 26 ± 1 | | | *1.6 ± 0.4* | | 24 ± 1 | 24 ± 1 | *-0.2 ± 0.9* | |
| BMI Z-score | | 0.1 ± 0.2 | 1.1 ± 0.2 \* | 1.0 ± 0.3 | | 1.5 ± 0.4 i | | | *0.5 ± 0.1* | | 0.9 ± 0.4 | 0.8 ± 0.4 b | *-0.1 ± 0.5* | |
| Δ Z-score birth weight - BMI | | -0.1 ± 0.2 | 1.6 ± 0.3 \* | 1.7 ± 0.4 | | - | | | *-* | | 1.4 ± 0.6 | - | *-* | |
| **Endocrine – Metabolic Variables** | | | | | | | | | | | | | | |
| Testosterone (nmol/ L) | | 1.0 ± 0.1 | 1.3 ± 0.1 \* | 1.3 ± 0.3 | | 0.9 ± 0.1 h | | | *-0.4 ± 0.2* | | 1.3 ± 0.2 | 1.0 ± 0.2 h | *-0.3 ± 0.2* | |
| SHBG (nmol/ L) | | 65 ± 4 | 32 ± 3 \*\*\* | 35 ± 5 | | 58 ± 9 i | | | *24 ± 6* | | 33 ± 4 | 32 ± 4 c | *-2 ± 2 g* | |
| FAI | | 1.7 ± 0.2 | 6.4 ± 0.8 \*\*\* | 6.8 ± 1.2 | | 3.0 ± 1.1 i | | | *-3.8 ± 1.1* | | 6.1 ± 1.6 | 3.9 ± 0.4 | *-2.2 ± 1.3* | |
| Fasting insulin (pmol/ L) | | 63 ± 5 | 70 ± 8 | 67 ± 12 | | 77 ± 9 | | | *11 ± 10* | | 55 ± 8 | 39 ± 6 c | *-16 ± 10 e* | |
| HOMA-IR | | 2.0 ± 0.2 | 2.1 ± 0.3 | 2.0 ± 0.4 | | 2.2 ± 0.2 | | | *0.2 ± 0.3* | | 1.7 ± 0.2 | 1.1 ± 0.2 c | *-0.6 ± 0.3  e* | |
| ALT (µkat/ L) | | 0.27 ± 0.01 | 0.25 ± 0.02 | 0.24 ± 0.02 | | 0.35 ± 0.05 h | | | *0.10 ± 0.05* | | 0.27 ± 0.03 | 0.24 ± 0.03 | *-0.03 ± 0.02 e* | |
| AST (µkat/ L) | | 0.32 ± 0.01 | 0.29 ± 0.02 | 0.27 ± 0.02 | | 0.28 ± 0.02 | | | *0.01 ± 0.01* | | 0.34 ± 0.04 | 0.28 ± 0.02 | *-0.05 ± 0.07* | |
| GGT (µkat/ L) | | 0.22 ± 0.01 | 0.24 ± 0.01 | 0.23 ± 0.02 | | 0.30 ± 0.04  h | | | *0.06 ± 0.03* | | 0.23 ± 0.01 | 0.20 ± 0.01  b h | *-0.03 ± 0.01 f* | |
| HDL-cholesterol (mmol/ L) | | 1.4 ± 0.0 | 1.3 ± 0.1 | 1.4 ± 0.1 | | 1.4 ± 0.1 | | | *0.0 ± 0.1* | | 1.4 ± 0.1 | 1.4 ± 0.1 | *0.1 ± 0.1* | |
| LDL-cholesterol (mmol/ L) | | 2.3 ± 0.1 | 2.2 ± 0.1 | 2.3 ± 0.2 | | 2.8 ± 0.2 h | | | *0.5 ± 0.2* | | 2.1 ± 0.2 | 2.1 ± 0.2 b | *0.0 ± 0.1 e* | |
| Triglycerides (mmol/ L) | | 0.6 ± 0.0 | 0.7 ± 0.0 | 0.7 ± 0.1 | | 0.8 ± 0.1 | | | *0.1 ± 0.1* | | 0.7 ± 0.1 | 0.8 ± 0.1 | *0.1 ± 0.1* | |
| HMW-adiponectin (mg/ L) | | 7.4 ± 0.7 | 4.7 ± 0.6 \*\* | 5.3 ± 1.0 | | 3.9 ± 0.5 | | | *-1.3 ± 0.8* | | 4.7 ± 0.9 | 7.1 ± 1.0 b | *2.4 ± 1 f* | |
| usCRP (nmol/ L) | | 6.4 ± 1.0 | 15.4 ± 3.5 | 11.0 ± 3.1 | | 25.6 ± 6.4 h | | | *14.5 ± 5.9* | | 14.9 ± 4.4 | 8.1 ± 3.5 b | *-6.8 ± 4.1 f* | |
| **Ovulatory function** ¥ | | | | | | | | | | | | | | |
| Total cycles | - | | - | | - | | 3 ± 0.4 | - | | - | | 6 ± 0.2 # | | - |
| Total ovulations | - | | - | | - | | 2 ± 0.5 | - | | - | | 4 ± 0.5 # | | - |
| **Body composition (DXA) ‡** | | | | | | | | | | | | | | |
| Bone mineral density (g/ cm2) | | - | 1.21 ± 0.02 | 1.18 ± 0.04 | | 1.18 ± 0.04 | | | *0.00 ± 0.01* | | 1.21 ± 0.02 | 1.19 ± 0.03 | *-0.02 ± 0.01* | |
| Lean mass (kg) | | - | 37 ± 1 | 37 ± 2 | | 38 ± 2 | | | *1 ± 1* | | 36 ± 1 | 37 ± 1 | *1 ± 1* | |
| Fat mass (kg) | | - | 25 ± 2 | 23 ± 2 | | 27 ± 2 j | | | *3 ± 1* | | 24 ± 3 | 23 ± 3 | *0 ± 2* | |
| Abdominal fat (kg) | | - | 6.5 ± 0.4 | 6.4 ± 0.6 | | 7.2 ± 0.7 j | | | *0.8 ± 0.3* | | 6.1 ± 0.7 | 5.9 ± 0.7 | *-0.2 ± 0.4 e* | |
| **Abdominal fat partioning (MRI)** | | | | | | | | | | | | | | |
| Subcutaneous fat (cm2) | | 98 ± 10 | 205 ± 24 | 194 ± 35 | | 220 ± 35 h | | | *26 ± 10* | | 194 ± 51 | 181 ± 42 | *-13 ± 28 e* | |
| Visceral fat (cm2) | | 29 ± 1 | 40 ± 4 | 37 ± 5 j | | 43 ± 5 | | | *6 ± 5* | | 32 ± 3 | 34 ± 4 | *2 ± 4* | |
| Hepatic fat (%) | | 11 ± 1 | 17 ± 1 \*\*\* | 16 ± 1 | | 18 ± 2 | | | *2 ± 3* | | 19 ± 1 | 10 ± 1 *d j* | *-9 ± 1 g* | |
| Central (hepato-visceral) fat | | 39 ± 2 | 57 ± 4 \*\* | 53 ± 5 | | 62 ± 5 | | | *9 ± 6* | | 51 ± 3 | 45 ± 5 *c* | *-7 ± 4 e* | |

Values are mean ± standard error (SEM).

BMI, body mass index; SHBG, sex hormone-binding globulin; FAI, free androgen index; HOMA-IR, homeostasis model assessment insulin resistance; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HMW, high molecular weight; usCRP, ultrasensitive C-reactive protein; DXA, dual X-ray absorptiometry; MRI, magnetic resonance imaging.

¥ Total numbers of cycles and ovulations over two periods of 12 consecutive weeks, during the second and fourth trimesters of the post-treatment year.

‡ Indicative DXA values in healthy adolescents, matched for age and height (N= 41): lean mass 35.1 ± 1.0 kg; fat mass 17.6 ± 1.4 kg (6).

\* P≤ 0.05; \*\* *P*≤ 0.01 and \*\*\* *P*≤ 0.001 between controls and girls with PCOS at baseline.

a no significant differences between randomized subgroups at start.

b *P* ≤ 0.05, c *P* ≤ 0.01 and d *P* ≤ 0.001 for differences between subgroups at 1 year.

e *P* ≤ 0.05, f *P* ≤ 0.01 and g *P* ≤ 0.001 for differences between subgroup changes (Δ) 0-1 year.

h *P* ≤ 0.05, i *P* ≤ 0.01 and j *P* ≤ 0.001 for differences within-subgroup changes from start.

# *P*< 0.0001 between subgropups differences in ovulatory function.

*P* values for differences between controls and girls with PCOS at baseline and between subgroups are adjusted for BMI.