

## **1. Results**

### **1.1 Effects of surfactin on related inflammatory responses of the serum in T2DM mice**

The serum levels of IL-1 $\beta$  in the T2DM group were significantly increased compared to the control group, while the levels of IL-1 $\beta$  in the surfactin-p group were significantly decreased than the T2DM group (Figure S3A). The levels of IL-1 $\beta$  in the surfactin-t group were not altered after HFD/STZ exposure (Figure S3A). The levels of TNF- $\alpha$  of the T2DM mice were noticeably higher than the control mice, while surfactin (in the surfactin-p and surfactin-t group) treatment noticeably lower this cytokine compared to the T2DM mice (Figure S3B). HFD/STZ treatment did not significantly alter the serum levels of NF- $\kappa$ B (Figure S3D). However, surfactin treatment (in the surfactin-t and surfactin-t group) significantly reduced the levels of NF- $\kappa$ B. In addition, surfactin supplementation did not alter the levels of IL-6 after HFD/STZ treatment (Figure S3C).

### **2.2. Effects of surfactin on related anti-oxidation of the serum in T2DM mice**

The serum levels of MDA of the T2DM mice were significantly increased than the control mice, while the levels of MDA were significantly decreased after surfactin treatment (in the surfactin-t and surfactin-t group) (Figure S4D). The serum activities of CAT in the surfactin-t group were dramatically enhanced compared to the T2DM group (Figure S4A). In addition, surfactin treatment did not alter SOD and GSH-Px activities compared to the T2DM mice (Figure S4B-C).

### **2.3 High performance liquid chromatography data**

Using the standard substance of surfactin, a standard curve was drawn with the concentration of surfctin as the abscissa and the peak area as the ordinate (Figure. S5). The formula for calculating the purity of surfactin samples is: measured concentration \* total volume / total sample weight. The concentration of surfactin sample 1-3 calculated according to the formula is as follows: 90.58%,

88.82, 86.68% (Figure S5). Therefore, the purity of surfactin was approximately 88.6%.

#### FIGURE CAPTIONS

**Figure S3.** Effects of surfactin on serum inflammatory cytokines in HFD/STZ-induced T2DM mice. The control group represents normal mice fed a basic diet, the T2DM group represents mice with T2DM induced by a 60% fat diet and 30 mg/kg body weight STZ, and the surfactin-p group represents T2DM mice treated with 80 mg/kg body weight surfactin before STZ injection, the surfactin-p group represents T2DM mice treated with 80 mg/kg body weight surfactin after STZ injection. The levels of IL-1 $\beta$  (A), TNF- $\alpha$  (E), IL-6 (F) and NF- $\kappa$ B (D). \* indicate significant differences at  $p < 0.05$ , \*\* indicate significant differences at  $p < 0.01$ .

**Figure S4.** Effects of surfactin on serum parameters associated with antioxidant in HFD/STZ-induced T2DM mice. The control group represents normal mice fed a basic diet, the T2DM group represents mice with T2DM induced by a 60% fat diet and 30 mg/kg body weight STZ, and the surfactin-p group represents T2DM mice treated with 80 mg/kg body weight surfactin before STZ injection, the surfactin-p group represents T2DM mice treated with 80 mg/kg body weight surfactin after STZ injection. The levels of CAT (A), SOD (B), GSH-Px (C) and MDA (D). \* indicate significant differences at  $p < 0.05$ , \*\* indicate significant differences at  $p < 0.01$ .

**Figure S5.** High performance liquid chromatography data. HPLC chromatogram of the standard of surfactin (A), HPLC chromatogram of surfactin sample 1 (B), HPLC chromatogram of surfactin sample 2 (C), HPLC chromatogram of surfactin sample 3 (D), standard curve of surfactin (E).

**Figure S1.** The western blot results for pancreas tissue. The control group represents normal mice fed a basic diet, the T2DM group represents mice with T2DM induced by a 60% fat diet and 30 mg/kg body weight STZ, and the surfactin group represents T2DM mice treated with 80 mg/kg body weight surfactin before STZ injection.

**Figure S2.** The western blot results for colon tissue. The control group represents normal mice fed a basic diet, the T2DM group represents mice with T2DM induced by a 60% fat diet and 30 mg/kg body weight STZ, and the surfactin group represents T2DM mice treated with 80 mg/kg body weight surfactin before STZ injection. The level of Caspase-1 (A), Cleaved-caspase-1(B), and Cleaved-caspase-1/Caspase-1. \* indicate significant differences at  $p < 0.05$ , \*\* indicate significant differences at  $p < 0.01$ .

Figure S1

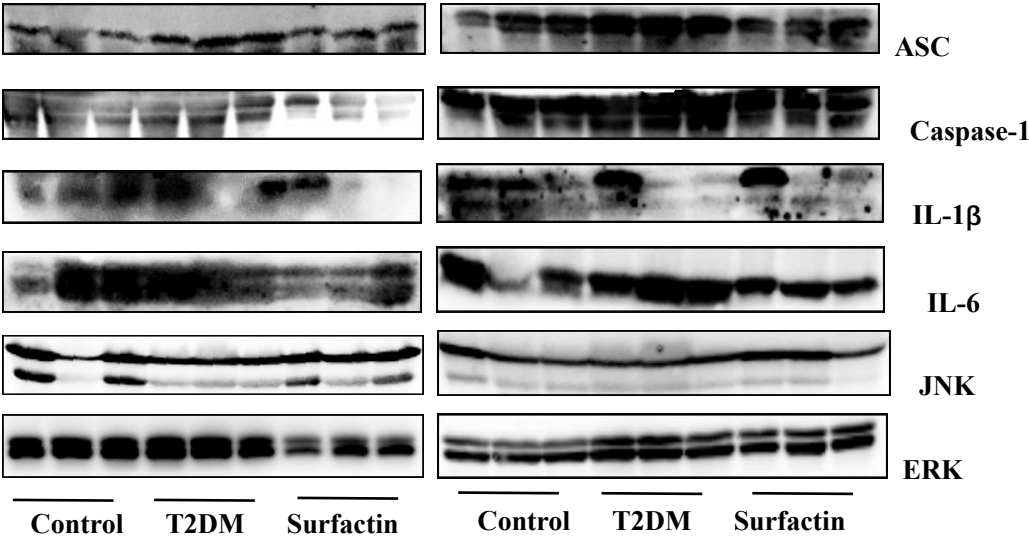


Figure S2

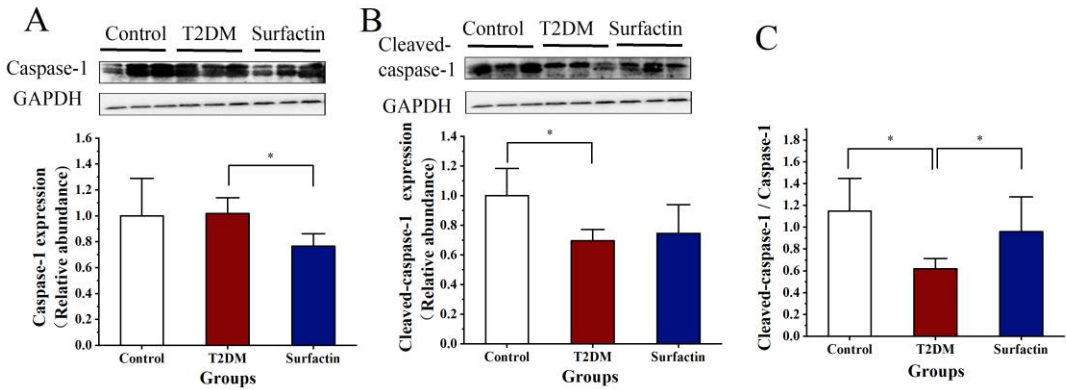


Figure S3

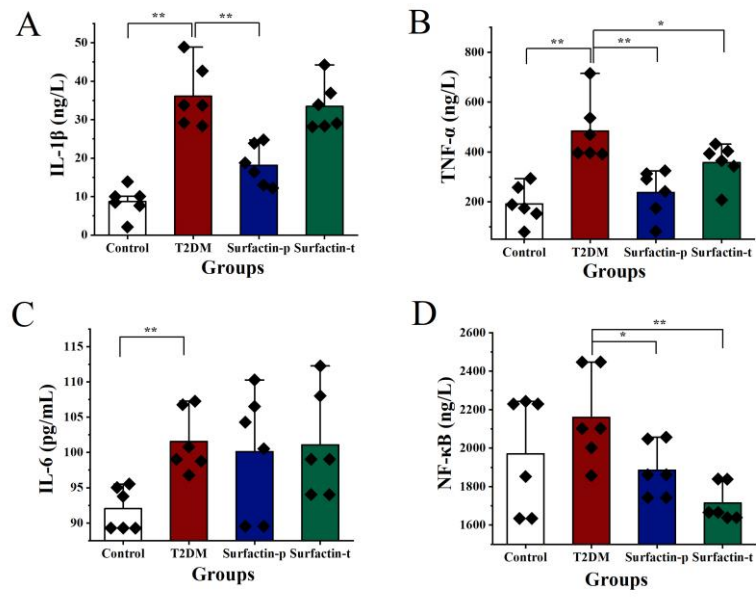


Figure S4

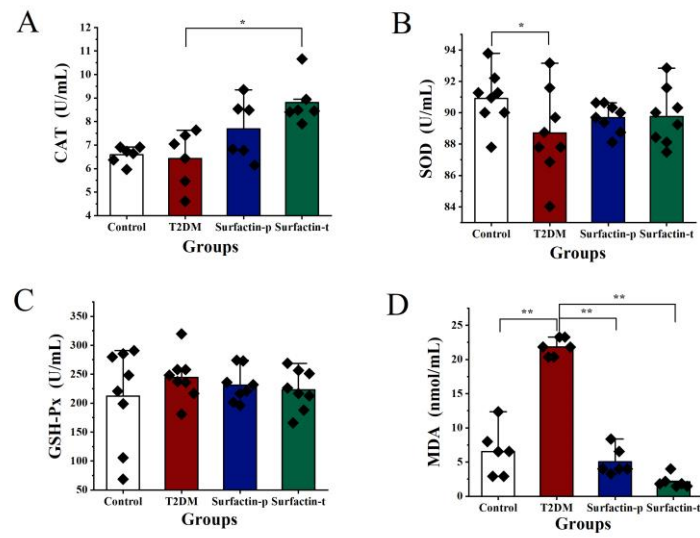
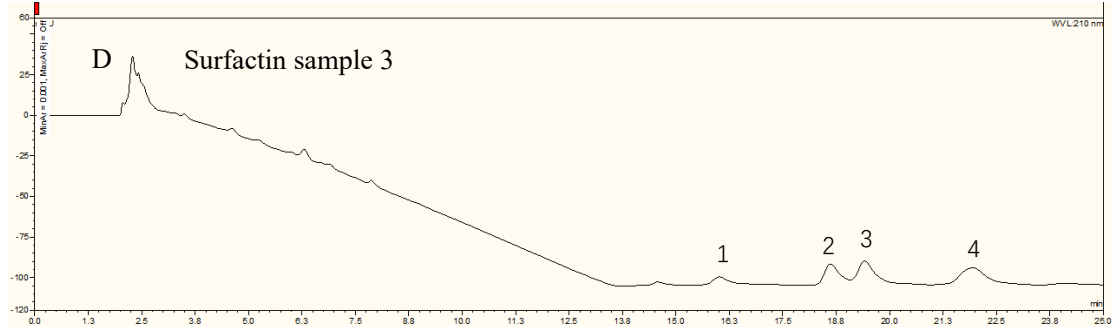
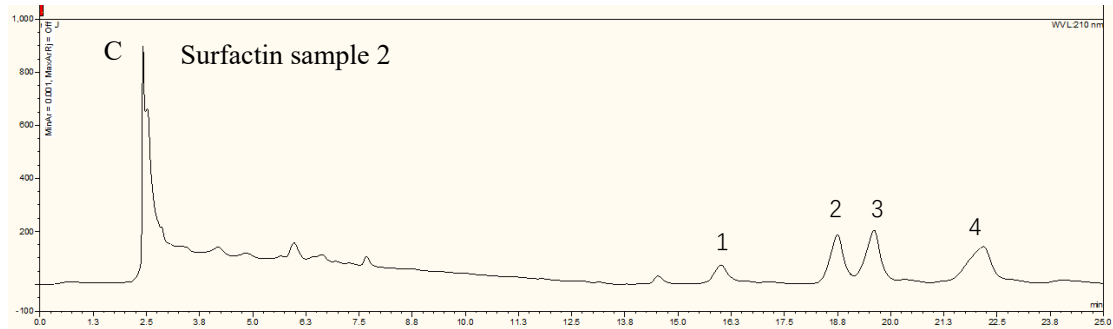
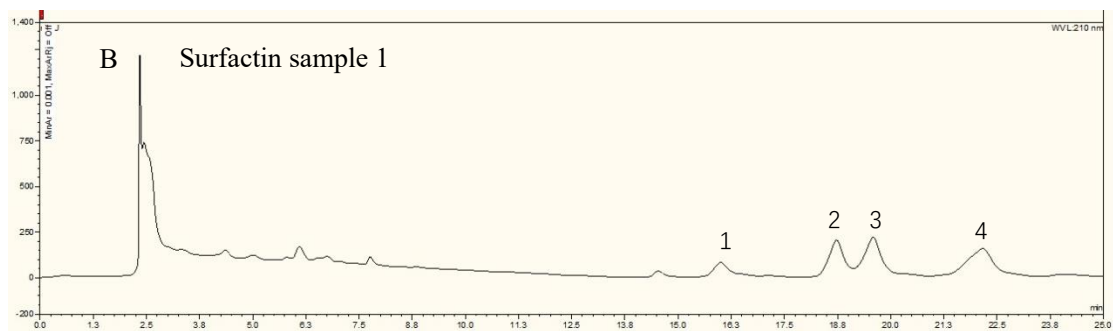
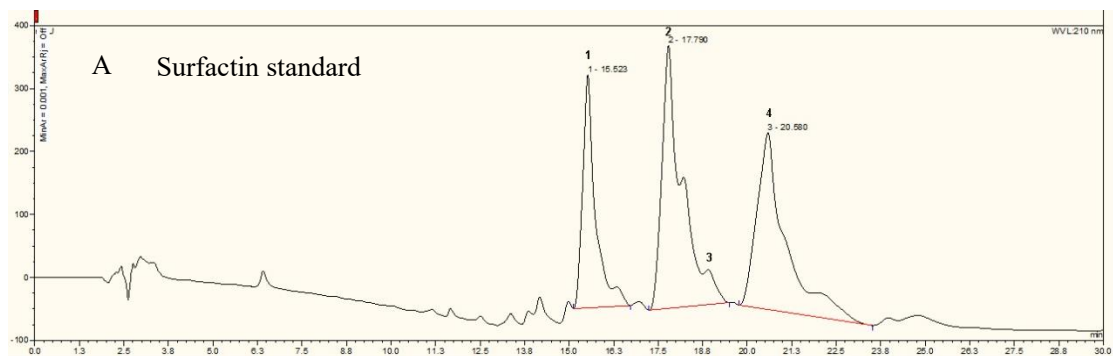
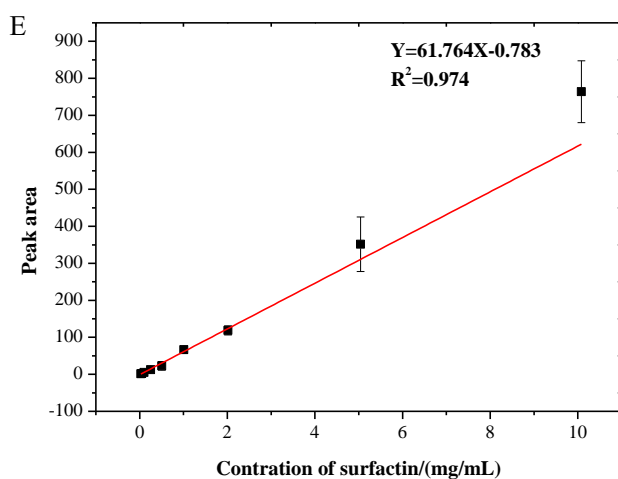


Figure S5





**Standard curve of surfactin**

**Table S1. The raw data for pancreas protein.**

| GAPDH  | Control | T2DM    | Surfactin  | Akt  | Control | T2DM    | Surfactin | p-Akt  | Control | T2DM    | Surfactin |
|--|---------|---------|------------|------|---------|---------|-----------|--------|---------|---------|-----------|
| Relative<br>grayscale  | 190608  | 152770  | 140139     |      | 190977  | 36147   | 161376    |        | 117906  | 87116   | 128720    |
|  | 174205  | 169887  | 199526     |      | 195973  | 315342  | 202685    |        | 76833   | 81161   | 141348    |
|  | 171894  | 172182  | 215613     |      | 200941  | 37142   | 207869    |        | 54489   | 84228   | 84022     |
|  |         |         |            |      |         |         |           |        |         |         |           |
| Calculation formula= Target protein/GAPDH (a), the final result = a/control a, the result is as follows: |         |         |            |      |         |         |           |        |         |         |           |
| P-Akt/Akt  | 1.47812 | 5.77009 | 1.90969520 | Akt  | Control | T2DM    | Surfactin | p-Akt  | Control | T2DM    | Surfactin |
|  | 0.93866 | 0.61620 | 1.66964842 |      | 0.91199 | 0.21536 | 1.048166  |        | 1.34803 | 1.24270 | 2.0016793 |
|  | 0.64922 | 5.42935 | 0.96774349 |      | 1.02396 | 1.68955 | 0.924639  |        | 0.96115 | 1.04110 | 1.5438235 |
|  |         |         |            |      | 1.06404 | 0.19634 | 0.877536  |        | 0.69080 | 1.06604 | 0.8492304 |
|  |         |         |            |      |         |         |           |        |         |         |           |
| GAPDH  | Control | T2DM    | Surfactin  | PI3K | Control | T2DM    | Surfactin | p-PI3K | Control | T2DM    | Surfactin |
| Relative   | 190608  | 152770  | 140139     |      | 12407   | 67825   | 9440      |        | 17665   | 10406   | 26335     |
|  | 174205  | 169887  | 199526     |      | 53258   | 43093   | 38304     |        | 21405   | 20841   | 40544     |
|  | 171894  | 172182  | 215613     |      | 45254   | 21475   | 36135     |        | 15911   | 41427   | 48548     |
|  |         |         |            |      |         |         |           |        |         |         |           |
| Calculation formula= Target protein/GAPDH (a), the final result = a/control a, the result is as follows: |         |         |            |      |         |         |           |        |         |         |           |
| p-PI3K/PI3K  | 2.93009 | 0.31573 | 5.74112192 | PI3K | Control | T2DM    | Surfactin | p-PI3K | Control | T2DM    | Surfactin |
|  | 0.82711 | 0.99528 | 2.17830107 |      | 0.30796 | 2.10053 | 0.318705  |        | 0.90237 | 0.66322 | 1.8297297 |
|  | 0.72356 | 3.96995 | 2.76489552 |      | 1.44644 | 1.20011 | 0.908284  |        | 1.19637 | 1.19445 | 1.9785167 |
|  |         |         |            |      | 1.24558 | 0.59009 | 0.792921  |        | 0.90125 | 2.34265 | 2.1923457 |

**Table S2 The raw data for colon protein.**

| GAPDH  | Control | T2DM  | Surfactin | Nf-κB | Control | T2DM    | Surfactin | TNF-α | Control | T2DM    | Surfactin |
|--|---------|-------|-----------|-------|---------|---------|-----------|-------|---------|---------|-----------|
| Relative   | 38995   | 46949 | 35867     |       | 25026   | 33960   | 13954     |       | 38943   | 64339   | 50118     |
| grayscale  | 36052   | 39078 | 36307     |       | 22526   | 37995   | 16659     |       | 71250   | 83437   | 13094     |
|  | 36171   | 41997 | 44725     |       | 20995   | 30067   | 10227     |       | 21533   | 18872   | 30670     |
| Calculation formula= Target protein/GAPDH (a), the final result = a/control a, the result is as follows: |         |       |           |       |         |         |           |       |         |         |           |
|  |         |       |           | Nf-κB | Control | T2DM    | Surfactin | TNF-α | Control | T2DM    | Surfactin |
|  |         |       |           |       | 1.04238 | 1.17486 | 0.631903  |       | 0.83914 | 1.15150 | 1.1741303 |
|  |         |       |           |       | 1.01484 | 1.57921 | 0.745256  |       | 1.66063 | 1.79408 | 0.3030397 |
|  |         |       |           |       | 0.94276 | 1.16283 | 0.371402  |       | 0.50022 | 0.37758 | 0.5762103 |
| GAPDH  | Control | T2DM  | Surfactin | IL-6  | Control | T2DM    | Surfactin | IL-1β | Control | T2DM    | Surfactin |
| Relative   | 38995   | 46949 | 35867     |       | 17102   | 63660   | 30738     |       | 40474   | 34656   | 36260     |
| grayscale  | 36052   | 39078 | 36307     |       | 32649   | 36077   | 13679     |       | 67287   | 21240   | 11608     |
|  | 36171   | 41997 | 44725     |       | 8934    | 45249   | 10054     |       | 9355    | 48053   | 21621     |
| Calculation formula= Target protein/GAPDH (a), the final result = a/control a, the result is as follows: |         |       |           |       |         |         |           |       |         |         |           |
|  |         |       |           | IL-6  | Control | T2DM    | Surfactin | IL-1β | Control | T2DM    | Surfactin |
|  |         |       |           |       | 0.82688 | 2.55649 | 1.615791  |       | 0.98445 | 0.70013 | 0.9588748 |
|  |         |       |           |       | 1.70743 | 1.74061 | 0.710343  |       | 1.77023 | 0.51552 | 0.3032468 |
|  |         |       |           |       | 0.46568 | 2.03139 | 0.423831  |       | 0.24530 | 1.08525 | 0.4585161 |
| GAPDH  | Control | T2DM  | Surfactin | IL-18 | Control | T2DM    | Surfactin | NLRP3 | Control | T2DM    | Surfactin |
| Relative   | 38995   | 46949 | 35867     |       | 165527  | 40629   | 89559     |       | 19382   | 19021   | 17285     |
|  | 36052   | 39078 | 36307     |       | 146681  | 125438  | 32430     |       | 68529   | 20434   | 71704     |
|  | 36171   | 41997 | 44725     |       | 39599   | 132918  | 81118     |       | 22547   | 46424   | 17824     |
| Calculation formula= Target protein/GAPDH (a), the final result = a/control a, the result is as follows: |         |       |           |       |         |         |           |       |         |         |           |
|  |         |       |           | IL-18 | Control | T2DM    | Surfactin | NLRP3 | Control | T2DM    | Surfactin |
|  |         |       |           |       | 1.35355 | 0.27594 | 0.796212  |       | 0.49354 | 0.40229 | 0.4785346 |
|  |         |       |           |       | 1.29735 | 1.02355 | 0.284820  |       | 1.88748 | 0.51923 | 1.9610653 |
|  |         |       |           |       | 0.34909 | 1.00920 | 0.578338  |       | 0.61896 | 1.09764 | 0.3957253 |
| GAPDH  | Control | T2DM  | Surfactin | TLR4  | Control | T2DM    | Surfactin | ASC   | Control | T2DM    | Surfactin |
| Relative   | 38995   | 46949 | 35867     |       | 46849   | 39187   | 43000     |       | 73532   | 62674   | 98405     |
|  | 36052   | 39078 | 36307     |       | 67494   | 39006   | 47937     |       | 92845   | 103764  | 52413     |
|  | 36171   | 41997 | 44725     |       | 28458   | 34352   | 37108     |       | 34838   | 111271  | 54938     |
| Calculation formula= Target protein/GAPDH (a), the final result = a/control a, the result is as follows: |         |       |           |       |         |         |           |       |         |         |           |
|  |         |       |           | TLR4  | Control | T2DM    | Surfactin | ASC   | Control | T2DM    | Surfactin |

|  |  |         |         |          |  |         |         |           |
|--|--|---------|---------|----------|--|---------|---------|-----------|
|  |  | 0.93366 | 0.64865 | 0.931694 |  | 1.04293 | 0.73833 | 1.5174455 |
|  |  | 1.45490 | 0.77570 | 1.026078 |  | 1.42436 | 1.46860 | 0.7984351 |
|  |  | 0.61142 | 0.63567 | 0.644788 |  | 0.53270 | 1.46539 | 0.6793811 |

**Table S3 The raw data for serum.**

| <b>GSP</b>     | <b>Control</b> | <b>T2DM</b> | <b>Surfactin-p</b> | <b>Surfactin-t</b> |
|----------------|----------------|-------------|--------------------|--------------------|
|                | 2539.08507     | 2455.47252  | 2850.73184         | 2751.91701         |
|                | 2455.47252     | 2957.14781  | 2204.63487         | 2645.50104         |
|                | 2212.23601     | 3147.17633  | 2508.6805          | 2698.70902         |
|                | 2105.82004     | 3420.8174   | 2318.65198         | 2995.15351         |
|                | 2584.69191     | 2751.91701  | 2493.47822         | 2364.25883         |
|                | 2288.24742     | 2789.92271  | 2599.89419         | 2736.71473         |
| <b>GHb</b>     | 680.5          | 715.5       | 470.5              | 665.5              |
|                | 703            | 848         | 590.5              | 573                |
|                | 633            | 875.5       | 423                | 665.5              |
|                | 733            | 678         | 595.5              | 823                |
|                | 685.5          | 773         | 773                | 800.5              |
|                | 633            | 900.5       | 640.5              | 665.5              |
| <b>Acrp30</b>  | 124.75224      | 69.22985    | 98.48358           | 86.84179           |
|                | 101.17015      | 78.48358    | 100.27463          | 104.45373          |
|                | 97.28955       | 85.94627    | 96.39403           | 88.93134           |
|                | 79.97612       | 79.67761    | 91.3194            | 99.0806            |
|                | 79.0806        | 92.21493    | 93.70746           | 112.81194          |
|                | 81.17015       | 92.81194    | 97.58806           | 93.11045           |
| <b>Insulin</b> | 6.88694        | 5.63581     | 6.95519            | 8.02434            |
|                | 7.02343        | 6.02252     | 7.27366            | 6.93244            |
|                | 6.36374        | 6.59122     | 6.11351            | 6.75045            |
|                | 7.22816        | 4.70314     | 6.36374            | 6.95519            |
|                | 6.56847        | 6.25        | 6.68221            | 7.50114            |
|                | 6.72771        | 6.72771     | 5.77229            | 5.34008            |
|                | 6.97793        | 5.13535     | 7.81961            | 6.45473            |



|                      |         |          |         |         |
|----------------------|---------|----------|---------|---------|
|                      | 6.65946 | 8.00159  | 7.68312 | 7.79686 |
| <b>HOMAIR</b>        | 1.79937 | 2.36852  | 1.99411 | 2.77514 |
|                      | 2.23056 | 2.19919  | 2.1143  | 2.54418 |
|                      | 1.75126 | 2.59648  | 2.10637 | 2.4005  |
|                      | 2.18139 | 2.36725  | 2.18185 | 2.28688 |
|                      | 2.08602 | 3.24154  | 1.60889 | 1.84126 |
|                      | 1.8617  | 2.5346   | 2.10637 | 2.15586 |
| <b>GLP-1</b>         | 2.54802 | 2.9774   | 3.5452  | 3.33898 |
|                      | 2.88701 | 2.9548   | 3.7226  | 3.30508 |
|                      | 2.71751 | 3.63277  | 3.17684 | 3.50847 |
|                      | 3.19209 | 3.51977  | 4.35593 | 3.16949 |
|                      | 2.71751 | 3.35028  | 4.27684 | 3.50847 |
|                      | 3.19209 | 2.81921  | 4.15593 | 3.33898 |
| <b>Blood glucose</b> | 5.87863 | 8.22506  | 7.42609 | 7.78139 |
|                      | 7.66452 | 8.45881  | 6.16849 | 9.04834 |
|                      | 6.19187 | 8.08527  | 7.78139 | 8.00112 |
|                      | 6.79028 | 10.12099 | 8.50136 | 7.39804 |
|                      | 7.14558 | 8.43806  | 7.34661 | 9.94642 |
|                      | 7.75849 | 8.21697  | 6.27134 | 7.75802 |
|                      | 7.19233 | 8.42506  | 7.18766 | 7.51491 |
|                      | 6.29004 | 10.02099 | 6.16849 | 7.34194 |