## Results (1343 words without figures/tables + their descriptions)

From the pre-processing of the subject datafile, 60 subjects remained of which 32 were IR and 28 were IS. The corresponding subject IDs were used to filter the proteome and metabolome data for the DAs, combined pathway analysis and network analysis. This resulted in 686 samples (316 IR and 370 IS) being included in the proteome data and 726 samples (425 IR and 302 IS) in the metabolome data. Moreover, the proteome data included 302 proteins annotated with HGNC symbols while the metabolome data was reduced from 724 metabolites to 323 metabolites after excluding those with no HMDB identifier specified.

After making the metagenomic and metabolomic datafiles consistent in order to run the integrative analysis, both datasets contained 49 subjects (26 IR and 23 IS) and 441 samples. This number of samples was reduced to 402 (200 IR and 202 IS) after excluding those with a PCA Axis1 value of less than -2.8. Despite excluding metabolites that had an abundance of 0 across many samples, the number of metabolites used in this integrative analysis remained the same (323 metabolites). Finally, filtering of the metagenomic data to only include sufficiently classified and abundant taxa, resulted in the phyloseq object containing 362 taxa.

### *Identification of differences in the gut microbiome of IR and IS subjects*

To determine whether differences in microbiome composition exist between the 2 groups, Bray-Curtis dissimilarities were illustrated using a PCoA. This PCoA demonstrated some clustering of the 2 groups, with 2 clusters being able to be distinguished per group (Figure 2). The separation seen in this plot was deemed to be significant by the PERMANOVA (p = 0.010) and was found to likely not be due to a significant difference in the variance of the groups by the multivariate analysis of the group dispersion homogeneity (p = 0.171). Nevertheless, there did exist a large variation in the absolute microbial abundances and absolute phyla abundances across both the IR and IS samples (Figure 3).



*Figure 2: Principal Coordinate Analysis (PCoA) based on Bray-Curtis dissimilarity illustrating the separation in the microbial composition of the insulin resistant (IR) and insulin sensitive (IS) samples. Variation explained by the corresponding principal coordinates are given in %.*



*Figure 3: bar plots depicting the variation in the absolute microbial abundance and in the absolute phyla abundances across the A) insulin resistant (IR) samples and B) the insulin sensitive (IS) samples.*

The mean abundance of each phyla in the different groups was investigated to determine which phyla were most likely to contribute to the distinction between the IR and IS metagenomes. Small differences in mean abundance between the 2 conditions were observed for the Actinobacteria, Firmicutes and Proteobacteria phyla, with the Firmicutes (Figure 4.C) and Proteobacteria (Figure 4.D) both being slightly less abundant and the Actinobacteria (Figure 4.A) slightly more abundant in the IR condition. However, only the differences in the Firmicutes and Proteobacteria phyla were significant (p< 0.0001 and p = 0.009, respectively).

More notable differences were seen for the remaining phyla. The Bacteroidetes were less abundant in the IR samples compared to the IS samples (Figure 4.B) while the Verrucomicrobia were more abundant in the IR samples (Figure 4.E). However, only the difference in Bacteroidetes was significant (p < 0.0001).

****

*Figure 4: box plots representing the differences in mean phylum abundance between the insulin resistant (IR) and insulin sensitive (IS) group. Significant p-values (p < 0.05) are indicated by \*\*\* = p < 0.0001, \*\* = p < 0.001 or \* = p < 0.01.*

To further investigate the taxa separating the groups, the top 20 taxa generated by the PERMANOVA were investigated in order to try identify the microbes contributing the most to the metagenomic differences between the 2 groups. The majority (70%) of these microbes belonged to the Firmicutes phylum.

Consistent with the Bacteroidetes mean abundance being lower in the IR group (Figure 4.B), the microbe least abundant in the IR condition compared to the IS condition was of the Bacteroidetes phylum (Figure 5). However, the microbe most abundant in the IR samples was found to belong to the Firmicutes phylum (Figure 5). This contradicted the observation of a lower Firmicutes mean abundance in the IR group (Figure 4.C).

3 of the 10 microbes more abundant in IR and none of those less abundant in IR were Ruminococcus. On the other hand, 2 out of the 10 microbes less abundant in IR and none of those more abundant in IR were Lachnospira. (Figure 5)



*Figure 56: bar plot of the taxa generated by the multivariate ANOVA with permutations (PERMANOVA) deemed to contribute the most to the separation between the insulin resistant and insulin sensitive groups. Taxa with negative values are less abundant in the IR condition and taxa with positive values are more abundant in the IS condition. Blue bars represent taxa belonging to the Bacteroidetes phylum, orange bars represent taxa belonging to the Firmicutes phylum and green bars represent taxa belonging to the Proteobacteria phylum.*

### *Identification of differences in the proteome and metabolome of IR and IS subjects*

From the DA using limma, 23 proteins were deemed to be significantly altered in the IR condition compared to the IS condition based on p-value. 13 of these proteins were more abundant in the IR subjects (logFC > 0) and 10, less abundant (logFC < 0) (Appendix A4). When a sufficient change was deemed to be a logFC > 0.50 or < -0.50, 3 of the significantly altered proteins remained: LPA and SHBG were both less abundant in the IR condition (logFC = -1.074 and -0.522, respectively) while APOC4 was more abundant in the IR condition (logFC = 0.624). Despite these proteins having significant p-values, it should be noted that none possessed a significant adjusted p-value.

On the other hand, the DA using MetaboDiff generated 40 metabolites whose abundances were significantly altered (p < 0.05) in the IR group compared to the IS group: 21 were less abundant (logFC < 0) and 19 more abundant in the IR condition (logFC > 0) (Appendix A5). Based on a logFC > 0.05 or < -0.05, 9 out of the 21 less abundant metabolites and 3 out of the 19 more abundant metabolites were sufficiently changed (Table 1). Unlike the results of the proteomic DA, 7 metabolites possessed a significant adjusted p-value with 5 also having a logFC suggesting a sufficient change in abundance (Table 1). The remaining 2 metabolites with a logFC > 0.05 or < -0.05 were HMDB02759 (logFC = -0.327) and HMDB00705 (logFC = 0.212).

*Table 1: table specifying the sufficiently less abundant (logFC < -0.50, blue cells) and sufficiently more abundant (logFC > 0.05, red cells) metabolites as determined by the differential analysis (DA) using MetaboDiff. The logFC and adjusted p-value are given for each metabolite. All metabolites listed were significantly changed in the IR condition compared to the IS condition based on p value (p < 0.05).*

|  |  |  |
| --- | --- | --- |
| Metabolite (metabolite type) | logFC | Adjusted p-value |
| LysoPE(0:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z)) (Lysophospholipid)  Super class = lipids and lipid-like molecules  Class = Glycerophospholipids | -1.336 | 0.005 |
| LysoPE(0:0/16:0) (Lysophospholipid)  Super class = lipids and lipid-like molecules  Class = Glycerophospholipids | -1.010 | 0.281 |
| LysoPE(P-16:0/0:0) (Phospho-ether lipid)  Super class = lipids and lipid-like molecules  Class = Glycerophospholipids | -1.089 | 0.042 |
| Ethylmalonic acid  Super class = lipids and lipid-like molecules  Class = Fatty acyls | -0.795 | 0.040 |
| 2-arachidonoylglycerol  Super class = lipids and lipid-like molecules  Class = endocannabinoids | -0.758 | 0.005 |
| 5-dodecenoic acid  Super class = lipids and lipid-like molecules  Class = Fatty acyls | -0.720 | 0.162 |
| MG(0:0/14:1(9Z)/0:0) (Monoacylglycerol)  Super class = lipids and lipid-like molecules  Class = glycerolipids | -0.683 | 0.026 |
| LysoPE(0:0/20:3(11Z,14Z,17Z)) (Lysophospholipid)  Super class = lipids and lipid-like molecules  Class = Glycerophospholipids | -0.591 | 0.158 |
| LysoPE(0:0/22:0) (Lysophospholipid)  Super class = lipids and lipid-like molecules  Class = Glycerophospholipids | -0.553 | 0.326 |
| Glutaric acid  Super class = organic oxygen compound  Class = organooxygen compound  Sub class = carbohydrate and carbohydrate conjugates | 0.556 | 0.326 |
| 2-trans,4-cis-Decadienoylcarnitine (Fatty acid ester)  Super class = lipids and lipid-like molecules  Class = fatty acyls | 0.604 | 0.106 |
| LysoPC(20:0/0:0) (Lysophospholipid)  Super class = lipids and lipid-like molecules  Class = Glycerophospholipids | 0.867 | 0.326 |

### *Identification of altered biological pathways involving these metabolomic and proteomic changes*

Combined pathway analysis of the proteome and metabolome data was done using PathVisio and MetaboAnalyst.

Out of all the 323 metabolites and 302 proteins run through PathVisio, only 15 metabolites and 25 proteins met the expression criterion (p < 0.05). The overrepresentation analysis of these compounds found 18 pathways to contain significantly more changed compounds than expected (Z-score > 1.96). All these pathways possessed 1-2 compounds that had significantly different levels in the IR subjects compared to in the IS subjects (p < 0.05). The majority of the significantly altered pathways involved metabolites that met the expression criteria but none contained both proteins and metabolites with a p < 0.05 (Table 2).

*Table 2: Significantly altered pathways (1.96 < Z-score < -1.96) and the associated significantly altered compounds (p < 0.05) as determined by combined pathway analysis using PathVisio. The common name for each metabolite is given while proteins are annotated using HGNC symbols. Arrows indicate the compound being more abundant in insulin resistance (****↑)*** *or less abundant in insulin resistance (****↓****), in comparison to the insulin sensitive condition.*

|  |  |  |
| --- | --- | --- |
| Pathway | Z-score | Proteins and Metabolites |
| Gastric acid production | 3.05 | **↑** Acetylcholine |
| Heroin metabolism | 3.05 | **↑** Pseudo-ChE |
| IL-1 signaling pathway | 3.05 | **↓** IL1RAP (CHECK) |
| Irinotecan pathway | 3.05 | **↑** BCHE |
| Monoamine GPCRs | 3.05 | **↑** Acetylcholine |
| Oligodendrocyte Specification and differentiation (including remyelination), leading to Myelin Components for CNS | 3.05 | **↓** Sphingomyelin (**d18:0/18:1(11Z)))** |
| Phosphodiesterases in neuronal function | 3.05 | **↑** L-Glutamate |
| Secretion of Hydrochloric Acid in Parietal Cells | 3.05 | ↑ Acetylcholine |
| Signal transduction through IL1R | 3.05 | ↓ IL1RAP |
| Sphingolipid pathway | 3.05 | ↓ Sphinganine |
| Structural Pathway of Interleukin 1 (IL-1) | 3.05 | **↓** IL1RAP |
| Thermogenesis | 3.05 | **↓** 2-Arachidonoylglycerol |
| Vitamin D-sensitive calcium signaling in depression | 3.05 | **↑** Acetylcholine |
| Ebola Virus Pathway on Host | 2.3 | **↓** GSN |
| Phosphatidylcholine catabolism | 2.3 | **↑** LysoPC(20:0/0:0)  **↓** Sphingomyelin (**d18:0/18:1(11Z)))** |
| PPAR Alpha Pathway | 2.3 | **↓** APOA1  **↓** PLTP |
| Senescence and Autophagy in Cancer | 2.3 | **↑** VTN  **↓** GSN |
| Glutathione metabolism | 1.97 | **↑** L-Glutamate  **↑** 5-Oxoproline |

MetaboAnalyst was unable to recognise one metabolite out of the 40 significantly altered metabolites (HMDB61112), hence, meaning 39 metabolites and 23 proteins were used in the corresponding joint pathway analysis. 6 pathways were found to be significantly changed (p < 0.05) in the IR subjects compared to the IS subjects. Like PathVisio, all these pathways concerned 1-2 significantly altered compounds, however, all were metabolites. Furthermore, the glutathione metabolism pathway as well as pathways associated with sphingolipids were present in the results of both programmes (Table 3).

*Table 3: List of significantly altered pathways ( p < 0.05) from the combined pathway analysis using MetaboAnalyst. The compounds whose abundance is significantly different between the insulin resistant (IR) and insulin sensitive (IS) subjects are given for each corresponding pathway. Arrows indicate compounds more abundant (*↑) *and less abundant (*↓) *in the IR condition.*

|  |  |  |
| --- | --- | --- |
| Pathway | p-value | Proteins and Metabolites |
| Porphyrin and chlorophyll metabolism | 0.019 | **↓** Biliverdin (isoform 1)  **↑** Biliverdin (isoform 2)  **↑** L-Glutamate |
|  |
| Glutathione metabolism | 0.021 | **↑** L-Glutamate  **↑** 5-Oxoproline |  |
|  |
| Sphingolipid metabolism | 0.022 | **↓** Sphinganine  **↓** Sphingomyelin (d18:0/18:1(11Z)) |  |
|  |
| Nitrogen metabolism | 0.040 | **↑** L-Glutamate |  |
| D-Glutamine and D-glutamate metabolism | 0.040 | **↑** L-Glutamate |  |
| Glycerophospholipid metabolism | 0.046 | **↓** 1-Acyl-sn-glycero-3-phosphocholine  **↑** Acetylcholine |  |
|  |

Since overrepresentation analysis can bury information concerning pathways that it deems to not be significantly altered, an extended network analysis of the significantly altered proteins was performed to uncover alterations in biological pathways that may have been lost in this analysis. The results revealed the human complement system and the complement and coagulation cascades pathways to possess the highest out-degree values: the first of these pathways having a value of 5 and the second a value of 4. Both of these pathways involved CFH and F10, with the 3 remaining proteins involved in the human complement system being C4A, APOA1 and VTN and the 2 remaining proteins in the complement and coagulation cascades pathway being SERPIND1 and C1QB.

### *Identification of associated microbes and metabolites*

The CCA used to determine which features best explain the covariation between the metabolomic and metagenomic data generated 13 microbes and 11 metabolites. 8 of these metabolites were monoacylglycerols with the remaining metabolites being arachidonic acid and 2-arachidonoylglycerol (2-AG). One of these monoacylglycerols (MG(0:0/14:1(9Z)/0:0)) as well as 2-AG were also shown to have significantly different abundances between the 2 groups by the DA (p < 0.05).

All 13 of the covariance-explaining microbes were Firmicutes. This coincides with the results of the PERMANOVA which showed that the majority of the taxa contributing most to the separation of the IR and IS metagenomes were Firmicutes (Figure 5). 6 of the 13 Firmicutes were Faecalibacterium prausnitzii, 3 were Oscillospira and 2 Coprococcus, with the remaining 2 taxa being Dorea and Blautia producta.

## Discussion

This study used systems biology approaches on iHMP T2D data to investigate the differences in the microbiome, host proteome and host metabolome between insulin sensitive and insulin resistant pre diabetics.The results were extended upon using functional and integrative analyses which allowed for interpretation within a biological context. As hypothesized, changes in all 3 of the investigated omics were observed. 23 of the 302 measured proteins and 40 of the 323 measured metabolites were seen to have significantly different abundances in the IR and IS group. Many of these changes were associated with immune function, energy homeostasis or lipid homeostasis and more than half of the significantly altered metabolites (23/40) were lipids/lipid-like molecules.

Concerning the metagenome, there existed a significant separation between the groups potentially due to differences in the abundance of certain phyla and microbes. Some of these distinguishing microbes were linked to the metabolomic differences, hence, suggesting an involvement in the host (patho)physiology.

General differences in the IR and IS microbiome significantly related to the *Bacteroidetes*, *Firmicutes* and *Proteobacteria* phylum which coincides with the fact that these phyla are 3 of the most abundant phyla in the human gut (<https://www.sciencedirect.com/science/article/pii/S0025619613008860?casa_token=PlE0swHVoegAAAAA:P_koV4iAk20zrFdcr6EtZesGyj7R_OaT86EqfhdX7EIVo5ZSD9XvfAjLavXlKkRs3ynq-_XlAf4>). All were lower in IR condition but the *Bacteroidetes* phylum had a considerably greater difference between the groups compared to the other 2 phyla. The *Bacteroidetes*/*Firmicutes* ratio has been found to be decreased in T2D (<https://www.sciencedirect.com/science/article/pii/S2213453013000451>). This coincides with the present results even though this ratio change was not very large. However, a decreased *Bacteroidetes*/*Firmicutes* has also been associated with obesity (<https://www.jci.org/articles/view/58109>). Since all participants included in the iHMP study had a BMI classifying them as obese, the ratio change seen in this study may not be as large as it could have been if all participants were lean. Furthermore, there existed a lot of variation in the absolute phyla abundances between the samples within each group. This could have potentially been caused by factors known to affect microbiome composition but which were not taken into account, such as ethnicity (<https://www-nature-com.ezproxy.ub.unimaas.nl/articles/s41591-018-0210-8>). This large variation may not only explain the minor change in the *Bacteroidetes*/*Firmicutes* ratio but also the observation of metagenomic separation within the IR and IS group themselves.

Nevertheless, investigation of the microbiome at OTU level rather than phylum level, produced results more consistent with previous literature.

For instance, the OTU more abundant in the IR condition that had the greatest discrepancy in abundance compared to the IS condition (*Oscillospira*), belongs to the *Firmicutes* phylum. This coincides with the finding that *Firmicutes* tend to be more abundant in IR individuals when compared to IS controls of similar BMI (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5209518/>). Hence, considering the present study population, it may be more beneficial to investigate differences at a level of classification more specific than phylum. For instance, an OTU classified as *Faecalibacterium prausnitzii* was found to be less abundant in the IR condition and to be the microbe contributing the 2nd most to the metagenomic separation of the groups. *F. prausnitzii* is a species of butyrate producing bacteria whose presence has been shown to improve insulin sensitivity by protecting against inflammation-induced insulin resistance (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6320976/>). Hence, a lower abundance of this species in IR subjects could potentially be an explanation for their insulin resistance. Moreover, almost half the OTUs that best explained the covariation between the metabolomic and metagenomic data were *F.prausnitzii*. This could suggest a possible link between the host metabolome and the presence of *F.prausnitzii*. For instance, one of the metabolites generated from the same analysis and which was deemed to be significantly less abundant in IR was 2-AG. Increased levels of 2-AG have been associated with administration of *Akkermansia muciniphila* ([*https://www.frontiersin.org/articles/10.3389/fnut.2020.00017/full*](https://www.frontiersin.org/articles/10.3389/fnut.2020.00017/full)), a bacteria that has been shown to cross-feed *F.prausnitzii* ([*https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6137959/*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6137959/)). Therefore, the lower abundance of *F.prausnitzii* in IR may explain the lower 2-AG levels since it may be an indication of low *A. muciniphila*. Yet, this conclusion requires quite a large jump from this study’s results and so should be taken with caution.

Concerning 2-AG function, it is an endocannabinoid able to bind receptors involved in thermogenesis (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4766995/>) as evidenced by the PathVisio results. Thermogenesis and others processes involved in energy homeostasis have been shown to be altered in IR states (<https://www.nature.com/articles/0801072.pdf>, <https://www.cell.com/cell-metabolism/pdf/S1550-4131(17)30220-6.pdf>).

In addition to thermogenesis, other pathways involved in energy homeostasis were also generated by the pathway analyses. One of these being the D-glutamine and D-glutamate metabolism pathway. This pathway involves L-glutamate which was found to be significantly more abundant in the IR subjects of this study. This change in abundance coincides with previous literature showing an association between high glutamate levels and T2D (<https://www.sciencedirect.com/science/article/pii/S0939475319302339?casa_token=vMnAX-4YzzAAAAAA:b0P0VKzlqbImBrQFFU8NzLcjDYYT41tjDz2rm1vLJ_A7GOfY0fHfouROrA-i8r8ZoowagT7n5G4>,), potentially due to the fact that it can be used as an alternative energy source (<https://www-sciencedirect-com.ezproxy.ub.unimaas.nl/science/article/pii/S2213858713701438>).

However, this adaptive increase in glutamate levels may result in decreased insulin secretion by accelerating beta-cell dysfunction through excessive NMDA receptor activation (<https://www.nature.com/articles/srep44120#:~:text=Taken%20together%2C%20these%20findings%20indicate,and%20apoptosis%20induced%20by%20hyperglycemia.>). This reduced insulin secretion along with the presence of insulin resistance in the IR group would result in a further decreased glucose uptake. This could propagate a more pronounced hypertriglyceridemia in the IR group as explained in the introduction. Higher levels of lipoproteins in the blood may be an explanation for the alterations in the abundance of some apolipoproteins seen in this study. For instance, APOC4 was seen to be more abundant in the IR condition compared to the IS condition. APOC4 is mainly found on VLDL (<https://www.jlr.org/content/44/7/1387.full.pdf>) and its overexpression has been associated with hypertriglyceridemia (<https://www.jlr.org/content/37/7/1510.full.pdf>). Furthermore, two apolipoproteins found on HDL, APOA1 (<https://www.nature.com/articles/labinvest201656>) and APOD (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2784685/#:~:text=Plasma%20ApoD%20is%20present%20mainly,risk%20of%20developing%20metabolic%20syndrome.>), were decreased in the IR subjects compared to the IS subjects. This could be evidence of low HDL levels which too have been previously associated with insulin resistance (<https://diabetes.diabetesjournals.org/content/diabetes/43/3/411.full.pdf?casa_token=pUBo9v-hdKcAAAAA:STuuisVaAFi2dgoWAdaRNkic2PTXSC28yUpGNOMxN9_KogBVHenPAz3ZO4NtmQEhoBjHqR073O-x0TVV>, <https://www.sciencedirect.com/science/article/pii/S0021915013007661?casa_token=PiDBd0nXSCUAAAAA:EjtO00oTruxRH0VNCPH51bLyJtlQDYlxDwnSyGBeDWV2ZBLA07Q7DFeGkyiMZ5Z-3DUjqLsOxFg>).  Other non-APO proteins have also been found on HDL particles, some of which are involved in the complement system such as C4A (<https://www.sciencedirect.com/science/article/pii/S2212963414000138#bib0125>). Like APOA1 and APOD, C4A was less abundant in the IR group compared to the IS group of this study and so could indicate the same dyslipidemia as these apolipoproteins. Any alterations in the levels of complement proteins obviously have consequences on immune function. This is demonstrated by the fact that multiple pathways generated by the overrepresentation analyses were associated with the immune system. However, all these pathways possessed only a small number of significantly altered proteins/metabolites compared to the total number of components involved in the pathway. Therefore, it is possible that these pathways are not altered enough to produce a noticeable difference between the IR and IS group. Yet, the low count of significantly altered compounds involved in the pathway analysis results could be more of an indication of a low coverage of the utilized tools rather than of a low-biological impact. Nevertheless, multiple tools were used to produce these results, hence, allowing for a more detailed representation of the potentially altered pathways. However, since these 2 analyses were unable to be performed simultaneously in one programme, the problem of coverage still exists within each of the tool sets. This problem could be minimized by the creation of one tool that encompasses the coverage of PathVisio, WikiPathways, KEGG and MetaboAnalyst.

Despite these issues in the interpretation of the pathway analyses, it should be noted that both pathways with the highest out-degree in the network analysis were associated with the complement system. This result could suggest more of an increased likelihood of marked changes in immune function compared to the results of the pathway analyses.

Another explanation for the possibly low biological effect of the observed metabolite and protein changes may have to do with the fact that baseline samples from which insulin sensitivity classification was determined, were not exclusively used. This could mean that some of the subjects in the IS group may have transitioned to become insulin resistant but were still classified as IS in the present study. Consequently, some of the changes between the classifications may not be as strong as they could have been if only the baseline samples were used. This may also explain the small change in the previously discussed *Bacteroidetes*/*Firmicutes* ratio.

Regardless of these limitations, this study did produce results consistent with the pathophysiology of insulin resistance especially in relation to the metabolomic and proteomic analyses. Due to time limitations and limitations within the field of metagenomics, analysis of the metagenomic data was more superficial. Integrative analysis of this data and the metabolomics data of both the IR and IS group would allow for a better understanding of the host-microbiome interactions driving the differences between these groups. Furthermore, any biological implications of the observed metagenomic changes could be better determined by the development of reliable functional analysis methods/tools.

## Conclusion

In conclusion, a separation in the metagenomes of IR and IS subjects was observed. This separation seemed to mainly be explained by changes in *Bacteroidetes* abundance. Direct association between the microbiome composition and the host metabolome was unable to be determined by the present analyses, however, certain OTUs and metabolites were found to be possible explanatory factors of the covariation between the datasets.

Concerning the proteomic and metabolomic data, the abundance of several metabolites and proteins was significantly different in the IR condition compared to IS. Many of these compounds were associated with the immune system, lipid homeostasis or energy homeostasis. This confirms the presently accepted knowledge concerning the pathophysiology of IR and could potentially be used to target future research towards biomarkers and therapeutic strategies for IR.