Urine Metabolomics by 1H-NMR Spectroscopy Indicates Associations between Serum 3,5-T2 Concentrations and Intermediary Metabolism in Euthyroid Humans

Maik Pietzner a, Georg Homuth b, Kathrin Budde a, Ina Lehmpfuhl d, Uwe Völker b, Henry Völzke c, Matthias Nauck a, Josef Köhrle d, Nele Friedrich a

a Institute of Clinical Chemistry and Laboratory Medicine, b Interfaculty Institute for Genetics and Functional Genomics, and c Institute for Community Medicine, University Medicine Greifswald, Ernst Moritz Arndt University, Greifswald, and d Institut für Experimentelle Endokrinologie, Charité-Universitätsmedizin Berlin, Berlin, Germany

Key Words
3,5-Diiodothyronine · Trigonelline · Urine metabolome · NMR spectroscopy · Thyroid hormone

Abstract
Context: 3,5-Diiodo-L-thyronine (3,5-T2) is a thyroid hormone metabolite which exhibited versatile effects in rodent models, including the prevention of insulin resistance or hepatic steatosis typically forced by a high-fat diet. With respect to euthyroid humans, we recently observed a putative link between serum 3,5-T2 and glucose but not lipid metabolism. Objective: The aim of the present study was to widely screen the urine metabolome for associations with serum 3,5-T2 concentrations in healthy individuals. Study Design and Methods: Urine metabolites of 715 euthyroid participants of the population-based Study of Health in Pomerania (SHIP-TREND) were analyzed by 1H-NMR spectroscopy. Multinomial logistic and multivariate linear regression models were used to detect associations between urine metabolites and serum 3,5-T2 concentrations. Results: Serum 3,5-T2 concentrations were positively associated with urinary levels of trigonelline, pyroglutamate, acetone and hippurate. In detail, the odds for intermediate or suppressed serum 3,5-T2 concentrations doubled owing to a 1-standard deviation (SD) decrease in urine trigonelline levels, or increased by 29–50% in relation to a 1-SD decrease in urine pyroglutamate, acetone and hippurate levels. Conclusion: Our findings in humans confirmed the metabolic effects of circulating 3,5-T2 on glucose and lipid metabolism, oxidative stress and enhanced drug metabolism as postulated before based on interventional pharmacological studies in rodents. Of note, 3,5-T2 exhibited a unique urinary metabolic profile distinct from previously published results for the classical thyroid hormones.

Introduction
During the last decade the concepts of thyroid hormone (TH) action underwent a sustainable gain in complexity [1–3]. New mechanisms of action concerning the classical TH: 1-thyroxine (T4) and 3,3′,5-triiodo-L-thyronine (T3) – became obvious, including biological effects of nonclassical TH such as tetraiodothyroacetic acid [3, 4] and 3,5-diiodo-L-thyronine (3,5-T2) [4], the putative...
deiodination product of T₃ in vivo. 3,5-T₂ exhibited remarkable metabolic effects when used as a pharmacological agent in animal studies. Administered to hypothyroid rats, 3,5-T₂ prevented and even reversed the severe consequences typically induced by a high-fat diet, like weight gain, insulin resistance or hepatic steatosis [5–7]. The responsible mechanisms include improvement of the blood lipid profile through the stimulation of β-oxidation of free fatty acids (FFA) and mitochondrial uncoupling, as well as depression of gluconeogenesis in hepatocytes [5, 8]. Therefore, 3,5-T₂ appears to act in a distinctively different mode compared to T₃.

Besides pharmacological studies in rodents, the knowledge about circulating 3,5-T₂ in humans is sparse. Early human studies in small and selected cohorts reported associations with chronic renal disease, liver cirrhosis or sep-sis [9–11]. Most of these early data stem from observational analyses and detection of 3,5-T₂ in human serum employing polyclonal antibody-based immunoassays which resulted in a wide spectrum of reported 3,5-T₂ serum concentrations spanning almost two orders of magnitude [for a review see 12]. Development and application of a monoclonal antibody-based chemiluminescent immunoassay recently revealed serum 3,5-T₂ concentrations in the range of 0.2–0.5 nM in healthy individuals [12]. These 3,5-T₂ values showed no correlation to serum T₄ or T₃ concentrations in healthy or T₄-substituted individuals, indicating that serum 3,5-T₂ has a metabolic fate independent of that related to T₄ and T₃. Using this assay we recently observed a putative link between serum 3,5-T₂ and glucose but not lipid metabolism in euthyroid humans [13].

Expanding these initial rodent experimental and human observational findings on the metabolic effects of 3,5-T₂, high-throughput techniques like proton nuclear magnetic resonance (¹H-NMR) spectroscopy can be used [14] to collect further information on the metabolic actions of 3,5-T₂. ¹H-NMR spectroscopy attempts to give a comprehensive view of small-molecule metabolites present in various biofluids, thus providing a more sensitive tool than classical clinical markers [15]. Urine, which is readily available, as a downstream product of human metabolism can especially mirror the impact of genetic determinants, environmental factors, personal behavior, nutrition and therapeutic intervention [16]. Despite these advantages, the use of metabolomics in the investigation of TH (metabolite) action is, to the best of our knowledge, limited to intervention studies in rodents [17–21] and, with the exception of a recent study on the relation between TH status and serum metabolites [22], completely lacking in humans. Therefore, the present study was designed to gain further insights into the metabolic profiles associated with circulating 3,5-T₂ by means of urine metabolomics in a large euthyroid study population.

**Material and Methods**

**Study Population**

SHIP-TREND is the second cohort of the Study of Health in Pomerania (SHIP), a population-based research project in West Pomerania, a rural region in North-East Germany [23]. A stratified random sample of 8,826 adults aged 20–79 years was drawn from population registries. Sample selection was facilitated by the centralization of local population registries in the Federal State of Mecklenburg-West Pomerania. The stratification variables were age, sex and city/county of residence. General baseline examinations were conducted between 2008 and 2012. Out of all the invitations, 4,420 individuals choose to participate (representing a 50.1% response rate). The study was approved by the local ethics committee and conformed to the principles of the declaration of Helsinki. For a specific SHIP-TREND subsample that encompasses 1,000 participants without self-reported diabetes who underwent an oral glucose tolerance test, a more extensive phenotyping was performed including, for example, additional laboratory measurements and metabolome analyses. This most comprehensively analyzed subsample of SHIP was chosen to ensure a maximum availability of clinically relevant information.

Of these participants, 995 were characterized by urine ¹H-NMR spectra. Furthermore, we excluded subjects with one of the following conditions (overlaps existed): low urinary creatinine concentrations (<2 mM, n = 50), missing values (n = 108) or values more than twice the standard deviation (SD) away from mean serum 3,5-T₂ concentrations (>2.13 nM, n = 15), use of thyroid medications (ATC code H03A or H03B, n = 98) or serum thyrotropin (TSH) levels outside the reference range (0.30–3.59 mU/l, n = 51). Ultimately, 715 subjects, aged between 21 and 81 years, were included in the present analysis.

**Measurements**

Each SHIP-TREND participant underwent standardized medical examinations, blood sampling and an extensive computer-aided personal interview. Data on sociodemographic characteristics and medical histories were collected. Waist circumference (WC) was measured by an inelastic tape between the lower rib margin and the iliac crest in the horizontal plane.

Blood and urine samples (fasting ≥8 h) were collected between 6:00 a.m. and 7:00 p.m. Blood samples were drawn from the cubital vein of subjects in the supine position. Both urine and blood samples were analyzed immediately or stored at −80°C. Serum TSH concentrations were measured using an immunoassay (Dimension VISTA, Siemens Healthcare Diagnostics, Eschborn, Germany) with a functional sensitivity of 0.005 mU/l. Serum 3,5-T₂ concentrations were measured with a recently developed monoclonal antibody-based chemiluminescence immunoassay [12]. The functional sensitivity of the assay was specified as 0.2 nM. The interassay variation was between 5.6 and 12.9%. The working range was declared as 0.2–10 nM 3,5-T₂. Urine creatinine concentrations were measured using the Jaffé method (Dimension VISTA, Siemens Healthcare Diagnostics).
Statistical Analysis

Continuous data are expressed as the median (1st; 3rd quartile), and nominal data as a percentage. For bivariate statistics the Wilcoxon rank sum test (continuous data) or $\chi^2$ test (nominal data) were used to compare men and women. Metabolite and metabolite ratios, as well as 3,5-T$_2$ concentrations, were log-transformed to achieve a normal distribution. Furthermore, metabolite/ratio levels were scaled according to their SD to facilitate comparison between metabolites exhibited a sex-specific phenotype, with mostly high-

Statistical Analysis

Continuous data are expressed as the median (1st; 3rd quartile), and nominal data as a percentage. For bivariate statistics the Wilcoxon rank sum test (continuous data) or $\chi^2$ test (nominal data) were used to compare men and women. Metabolite and metabolite ratios, as well as 3,5-T$_2$ concentrations, were log-transformed to achieve a normal distribution. Furthermore, metabolite/ratio levels were scaled according to their SD to facilitate comparison between associations. Since about a third of the study population exhibited 3,5-T$_2$ concentrations below the detection limit of the used assay (0.2 nM) but distinctly higher than blank values of the standard curve, subjects were subdivided into three groups according to their 3,5-T$_2$ concentrations: <0.2 nM (n = 255) but distinct from zero, 0.2–0.33 nM (n = 230) and >0.33 nM (n = 230). Subsequently, multinomial regression models with metabolite/ratio levels as exposures and 3,5-T$_2$ groups as outcome were performed. After the exclusion of participants with zero values for each metabolite/ratio and 3,5-T$_2$ concentrations below 0.2 nM, multivariate linear models with 3,5-T$_2$ concentrations as the continuous outcome were performed. All models were adjusted for age, sex and WC, as well as TSH concentrations outside the reference range (n = 757; n = 267 with serum 3,5-T$_2$ <0.2 nM) was tested to analyze the influence of an altered thyroid state. A p value <1.1 × 10^{-4} (Bonferroni correction) for metabolites or <4.6 × 10^{-5} (Bonferroni correction) for metabolite ratios was considered as statistically significant. Statistical analyses were performed using SAS version 9.3 (SAS statistical software, SAS Institute Inc., Cary, N.C., USA) and R 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

The general characteristics for men and women are summarized in table 1. Women were more often never smokers and had lower values of WC, whereas serum TSH levels were higher compared to men. No sex-specific differences regarding age or 3,5-T$_2$ concentrations became obvious. Creatinine-standardized urine metabolites exhibited a sex-specific phenotype, with mostly higher values among women (online suppl. table S1).

Multinomial logistic regression models revealed several metabolites, including pyroglutamate, hippurate, acetone, formate and trigonelline, significantly associated with 3,5-T$_2$ (fig. 1, 2; table 2). In detail, the odds for sup-

### Table 1. General characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Male (n = 351)</th>
<th>Female (n = 364)</th>
<th>p$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>50.2 (38.6; 60.8)</td>
<td>48.1 (40.2; 59.5)</td>
<td>0.70</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td>Never</td>
<td>32.4</td>
<td>52.5</td>
</tr>
<tr>
<td></td>
<td>Former</td>
<td>43.2</td>
<td>28.0</td>
</tr>
<tr>
<td></td>
<td>Current</td>
<td>24.4</td>
<td>19.5</td>
</tr>
<tr>
<td>Physical activity, %</td>
<td>&gt;1 h/week</td>
<td>50.7</td>
<td>51.9</td>
</tr>
<tr>
<td></td>
<td>&lt;1 h/week</td>
<td>49.3</td>
<td>48.1</td>
</tr>
<tr>
<td>WC, cm</td>
<td>94.0 (86.2; 102.3)</td>
<td>81.5 (73.7; 90.1)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TSH, mU/l</td>
<td>1.11 (0.79; 1.47)</td>
<td>1.29 (0.91; 1.76)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3,5-T$_2$, nM</td>
<td>0.24 (0.20; 0.38)</td>
<td>0.25 (0.20; 0.36)</td>
<td>0.91</td>
</tr>
<tr>
<td>Urine creatinine, mM</td>
<td>12.5 (7.1; 17.0)</td>
<td>6.5 (4.0; 11.0)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data are expressed as median (25th; 75th percentile).

$^1$ The Wilcoxon rank sum test for continuous and the $\chi^2$ test for categorical data were used for comparison.
pressed (<0.2 nM) or intermediate (0.2–0.33 nM) serum 3,5-T₂ concentrations doubled owing to a 1 SD decrease in logarithmic trigonelline levels. Furthermore, 29–50% increased odds for suppressed or intermediate serum 3,5-T₂ concentrations were related to a 1 SD decrease in urine pyroglutamate, acetone and hippurate levels (fig.1, 2; table 2). Multivariate linear models confirmed these findings, even if only the positive association with trigonelline reached the corrected statistical significance (fig. 3).

Adjustment for TSH levels in multinomial logistic and linear regression models did not change the above-mentioned associations (fig. 3; online suppl. fig. S1). Interestingly, the inclusion of subjects with extreme serum 3,5-T₂ or TSH concentrations outside the reference range led to a general loss of significance (data not shown). Regardless, the associations towards trigonelline and hippurate remained significant.

Fig. 1. Results for 3,5-T₂ from multinomial logistic regression (ref. >0.33 nM) models adjusted for age, sex and WC. p values for the suppressed (<0.20 nM) or intermediate (0.20–0.33 nM) group are colored blue and orange, respectively. Each line and column represents one metabolite; the diagonal contains results for single metabolites divided into triangles for each hormone, all other boxes represent the respective ratio; thick-framed triangles indicate significantly associated metabolite ratios regarding trigonelline (fig. 3).

Adjustment for TSH levels in multinomial logistic and linear regression models did not change the above-mentioned associations (fig. 3; online suppl. fig. S1). Interestingly, the inclusion of subjects with extreme serum 3,5-T₂ or TSH concentrations outside the reference range led to a general loss of significance (data not shown). Regardless, the associations towards trigonelline and hippurate remained significant.
Table 2. Significant associations between urinary metabolites and serum 3,5-T₂ concentrations

<table>
<thead>
<tr>
<th>Metabolites per SD decrease</th>
<th>n</th>
<th>SD</th>
<th>OR (95% CI)¹ (ref. &gt;0.33 nM)</th>
<th>n</th>
<th>SD</th>
<th>β (95% CI)²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;0.2 nM p</td>
<td></td>
<td></td>
<td>0.2–0.33 nM p</td>
<td></td>
</tr>
<tr>
<td>Pyroglutamate</td>
<td>656</td>
<td>0.24</td>
<td>1.36 (1.11; 1.65) &lt;0.01</td>
<td>428</td>
<td>0.26</td>
<td>–0.059 (–0.102; –0.016) &lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Hippurate</td>
<td>715</td>
<td>0.84</td>
<td>1.44 (1.19; 1.76) &lt;0.01</td>
<td>460</td>
<td>0.83</td>
<td>–0.054 (–0.098; –0.011) 0.01</td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>561</td>
<td>0.73</td>
<td>1.29 (1.04; 1.59) 0.02</td>
<td>363</td>
<td>0.69</td>
<td>–0.057 (–0.104; –0.010) 0.02</td>
<td></td>
</tr>
<tr>
<td>Trigonelline</td>
<td>665</td>
<td>0.88</td>
<td>2.46 (1.96; 3.08) &lt;0.01</td>
<td>434</td>
<td>0.87</td>
<td>–0.152 (–0.194; –0.112) &lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

¹ All metabolites were in relation to creatinine. ref. = Reference tertile. ² p < 0.05/47 = 1.1 × 10⁻³.
³ Multinomial logistic regression models adjusted for age, sex and WC. ² Multivariate linear regression models adjusted for age, sex and WC.

Fig. 2. Box plots of levels of urine pyroglutamate, hippurate, acetone and trigonelline by 3,5-T₂ concentration. The black triangles indicate group means. Asterisks (*) indicate a significant odds ratio compared to the highest group from the multinomial logistic regression analysis (see table 2).
Discussion

The present study screened the human urine metabolite profile assessed by $^1$H-NMR spectroscopy for associations towards serum 3,5-T$_2$ concentrations. As discussed below, our results regarding trigonelline, pyroglutamate or acetone support and extend previously observed associations between 3,5-T$_2$ and glucose or lipid metabolism as demonstrated by various animal models [4, 5, 8, 25–29]. Furthermore, we observed thyromimetic, but TSH independent, associations regarding hippurate and pyroglutamate, as observed in recent animal studies [17, 19].

Among all associated metabolites, trigonelline exhibited the strongest association with 3,5-T$_2$ and remains significant even after adjustment for serum or urine glucose levels, possibly confounding the association. The origin of urine trigonelline could either be exogenous, as food ingredients, or endogenous as a product of niacin metabolism [30, 31]. Administration of trigonelline reduced blood glucose levels by improving insulin sensitivity after

![Fig. 3. Results for 3,5-T$_2$ colored according to p values form multivariate linear regression models adjusted for age, sex and WC (model 1; blue) and further for serum TSH levels (model 2; orange). Each line and column represents one metabolite; the diagonal contains results for single metabolites, all other boxes represent the respective ratio; light blue frames indicate significant metabolites: $p_{\text{meta}} = 0.05/47 = 1.1 \times 10^{-3}$; dark blue frames indicate significant ratios: $p_{\text{all}} = 0.05/1,082 = 4.6 \times 10^{-5}$ (accordingly corrected for multiple testing). Ratios with creatinine were not included in the analysis (colored gray), since all metabolites were normalized on creatinine.](image)
experimental induction of type 2 diabetes mellitus [32–34]. Similar effects were reported for 3,5-T2 when used as a pharmacological agent to prevent insulin resistance forced by a high-fat diet [5]. Further animal studies [27, 35, 36] confirmed the antidiabetic effect of both trigonelline and 3,5-T2, revealing protective effects on impaired kidney function and structure, which represents a severe sequela of type 2 diabetes mellitus. Taken together, 3,5-T2 and trigonelline were linked to glucose metabolism, exhibiting the potential to improve glucose homeostasis in the case of diminished insulin sensitivity. Since a decrease in 3,5-T2 concentrations was associated with a decrease in urine trigonelline levels, a mutual interaction or interdependency appears possible. In this context previous reports on inhibition of T3 production by administration of fenugreek seed extracts in rodents are of interest. These trigonelline-rich extracts inhibit T3 production from T4 concomitant to decreasing hepatic superoxide dismutase activity, but leaving lipid peroxidation and catalase activity unaltered [37], thus supporting links between TH status, glucose metabolism and trigonelline, which still need to be unraveled in detail.

Glucose metabolism was further related to 3,5-T2 via stimulation of glucose-6-phosphate dehydrogenase (G6PD) [38], a key enzyme of the pentose phosphate pathway. G6PD catalyzes the conversion of D-glucose-6-phosphate to 6-phospho-D-glucono-1,5-lactone, thereby producing nicotinamide adenine dinucleotide phosphate (NADPH). NADPH in turn is necessary for the reduction of oxidized glutathione, to restore intracellular levels of reduced glutathione after response to oxidative stress. Beside this redox cycle, glutathione synthesis is assured by the γ-glutamyl cycle, which at least in astrocytes [39] was assumed to be stimulated by TH. The γ-glutamyl cycle involves the formation of pyroglutamate (5-oxoproline), which could be converted to glutamate and hence integrated in glutathione synthesis, but its conversion depends on glutathione synthase activity. Depressed glutathione synthase activity leads to a rise of pyroglutamate excretion in blood and subsequently in urine [40]. In this context, the observed positive association between 3,5-T2 and urine pyroglutamate levels might point towards a link between 3,5-T2 and antioxidant defense, supporting observations from a recent proteomics study [29]. Shifts in hepatic cysteine flux, altered TH homeostasis and lipid metabolism have recently been observed in a study on the hepatic profile in mice with suboptimal hepatic and systemic expression of enzymes involved in cellular redox regulation due to mild selenium deficiency [20].

Acetone is a ketone body derived by a spontaneous decarboxylation of acetoacetate, which in turn is derived from acetyl-CoA. Generally, acetyl-CoA serves as substrate for the tricarboxylic acid cycle by condensing with oxaloacetate. In a state of low oxaloacetate levels, acetyl-CoA is redirected to ketogenesis. This displays a switch in energy metabolism from glucose to FFA utilization. Urine ketone body levels were considered as markers for mitochondrial β-oxidation of FFAs [41]. The positive association between 3,5-T2 concentrations and urine acetone levels observed in our study is in concordance with several animal studies [8, 25, 26] showing rapid enhanced FFA transportation and subsequent oxidation in the mitochondria of skeletal muscle and hepatocytes of hypothyroid rats treated with 3,5-T2. As the molecular target carnitine palmitoyltransferase-1 (CPT1) was identified, which constitutes the main gateway for FFAs to mitochondria [8, 25]. Of note, related enzymes, including CPT1, were reported to be altered by 3,5-T2 treatment in the liver of rats fed with a high-fat diet [29]. Furthermore, enhanced ketogenesis following TH metabolite administration was even observed in two previous studies, where 3-iodothyronamine (3-T1AM) treatment results in elevated serum and urine ketone bodies [21, 42]. This observation might support the hypothesis that 3,5-T2 might be (one of) the precursor(s) of biosynthesis of 3-T1AM [12, 43, 44]. Consequently, it should be considered to expand the concept of TH action on (lipid) metabolism to metabolically active TH derivatives originating from the classical TH T4 and T3 by further deiodination and decarboxylation in target tissues such as liver, skeletal muscle and adipose tissue.

In concordance with this hypothesis, we observed an association with urine levels of hippurate, which were shown to be elevated in the case of experimental induction of hypothyroidism in rats [19]. Since previous work [12] suggested unaltered serum 3,5-T2 concentrations in hypothyroidism, a decreased ratio of the putative precursor T4 (T3) and 3,5-T2 could account for this observation. However, the relation between serum fT4 as well as TSH and 3,5-T2 concentrations in humans is not yet completely understood. Moreover, in the present study the inclusion of subjects with abnormal serum 3,5-T2 or TSH concentrations led to weaker associations, suggesting differing roles of serum 3,5-T2 on intermediary metabolism in different thyroid states. It is worth noting that, besides hippurate, 3,5-T2 exhibited a unique associated urinary metabolic profile in comparison with TSH and fT4 [19].

Our study has some strengths and some potential limitations. Metabolomics is a powerful tool for endocrine
research, since it has the capability of jointly capturing versatile influences, like genetics or health behavior, in intermediate phenotypes. These intermediate phenotypes even enable the analysis of effects of moderate endocrine actors. In contrast to the previously performed intervention studies on animals, the present study is limited by the cross-sectional and agnostic design, whereby no prediction of time courses and detection of intervention effects are possible. Moreover, the functional sensitivity of the assay used to measure serum 3,5-T₂ represented a strong limitation in the statistical analysis, since one third of study participants (n = 255) exhibited concentrations below this detection limit. Our findings should therefore be regarded as hypothesis generating. It is widely accepted that ¹H-NMR spectroscopy is hampered by lower sensitivity in comparison with chromatographic coupled mass spectrometry. Hence, we expect lower insight into the role of serum 3,5-T₂ in human metabolism.

**Acknowledgements**

This work was funded by grants from the German Federal Ministry of Education and Research (BMBF, grants 01ZZ0403, 01ZZ0103, 01GI0883), the Ministry for Education, Research and Cultural Affairs, as well as the Ministry of Social Affairs of the Federal State of Mecklenburg-West Pomerania. This work is also part of the research project Greifswald Approach to Individualized Medicine (GANI_MED). The GANI_MED consortium is funded by the Federal Ministry of Education and Research and the Ministry of Cultural Affairs of the Federal State of Mecklenburg-West Pomerania (03IS2061A). The project was conducted within the framework of the DFG SPP 1692 'Thyroid Trans Act' (DFG WA 1328/5-1, KO 922/17-1 and VO 955/12-1) and the DFG GRK 1208-2 (TP 3 to J.K.).

**Disclosure Statement**

The authors declare no conflicts of interest.

**References**


3,5-T₂ and the Urine Metabolome


