Klinika Endokrynologii i Chorób Metabolicznych
Instytut „Centrum Zdrowia Matki Polki" w Łodzi

lek. Katarzyna Adamczewska

Wpływ czynników pozatarczycowych na modulację wydzielania TSH u dzieci ze szczególnym uwzględnieniem czynników infekcyjnych, hormonów szlaku oreksygenicznego i hormonów tkanki tłuszczowej

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Spis treści

1. Wykaz publikacji stanowiących pracę doktorską

1. **Adamczewska K,** Adamczewski Z, Stasiak M, Lewiński A, Stawerska R. Transient Hyperthyrotropinemia in Outpatient Children with Acute Infections of the Respiratory System. Int J Environ Res Public Health. 2021 Apr 13;18(8):4115. **[IF 3,39; MEiN 70]**

2. **Adamczewska K,** Adamczewski Z, Łupińska A, Lewiński A, Stawerska R. Strong Positive Correlation between TSH and Ghrelin in Euthyroid Non-Growth Hormone-Deficient Children with Short Stature. Molecules. 2020 Aug 27;25(17):3912. **[IF 4,412; MEiN 100]**
3. **Adamczewska K,** Adamczewski Z, Lewiński A, Stawerska R. Leptin does not stimulate TSH secretion in obese short children. Front. Endocrinol. 2022; 13:838881. **[IF 5,555; MEiN 100]**

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2. STRESZCZENIE

Wpływ czynników pozatarczycowych na modulację wydzielania TSH u dzieci ze szczególnym uwzględnieniem czynników infekcyjnych, hormonów szlaku oreksygenicznego i hormonów tkanki tłuszczowej

Publikacja Nr 1.

Adamczewska K, Adamczewski Z, Stasiak M, Lewiński A, Stawerska R.

Transient Hyperthyrotropinemia in Outpatient Children with Acute Infections of the Respiratory System.

Int J Environ Res Public Health. 2021 Apr 13;18(8):4115.

Wstępna diagnostyka chorób tarczycy często opiera się wyłącznie na oznaczeniu stężenia hormonu tyreotropowego (TSH). Wykrycie stężenia TSH poza zakresem referencyjnym, bywa przyczyną przeprowadzania kosztownej i czasochłonnej diagnostyki. Obserwacje wskazują, że u znacznej części pacjentów ze stwierdzonym początkowo podwyższonym stężeniem tego hormonu nie stwierdza się choroby tarczycy. Celem pracy była ocena częstości występowania powszechnie znanego zjawiska przemijającej hipertyrotropinemii u ogólnie zdrowych dzieci w przebiegu ostrych infekcji układu oddechowego. Badaniem objęto łącznie 94 dzieci (49 chłopców i 45 dziewcząt) w wieku od 2,2 do 17,3 lat, które zgłosiły się do lekarza pierwszego kontaktu z powodu ostrej infekcji dróg oddechowych. Oceniano morfologię krwi obwodowej, białko C-reaktywne (CRP), TSH i wolnej tyroksyny (FT_4). Oznaczenia były wykonywane następnego dnia po wizycie u lekarza (wizyta wstępna) i $\geq$2 tygodnie po wyzdrowieniu. Wśród przebadanych dzieci podwyższone wartości TSH stwierdzono u około 10% pacjentów. Wartości te powróciły do normy po wyzdrowieniu. Ponadto analiza prospektywna wykazała istotne (ale w zakresie wartości referencyjnych) zwiększenie wartości stężenia TSH u około 65% wszystkich dzieci. Podczas wizyty kontrolnej stężenia były niższe. Pozwoliło to na wyciągnięcie istotnego klinicznie wniosku, iż w przypadku stwierdzenia umiarkowanie podwyższonej wartości TSH u dzieci w przebiegu infekcji dróg oddechowych konieczne jest wykonanie badania kontrolnego stężenia tego hormonu po wyzdrowieniu.

Publikacja Nr 2.

Adamczewska K, Adamczewski Z, Łupińska A, Lewiński A, Stawerska R.

Strong Positive Correlation between TSH and Ghrelin in Euthyroid Non-Growth Hormone-Deficient Children with Short Stature. Molecules. 2020 Aug 27;25(17):3912.

Procesy wzrostu u dzieci zależą od prawidłowego funkcjonowania niektórych hormonów i czynników wzrostu. Ostatnie badania udowadniają dodatnią korelację między greliną a TSH u pacjentów z nadczynnością i niedoczynnością tarczycy. Ponadto zaobserwowano, że u szczurów z niedoczynnością tarczycy z wysokim stężeniem greliny wydzielanie hormonu wzrostu (GH) i insulinopodobnego czynnika wzrostu I (IGF-I) było zahamowane. W niniejszej pracy przeanalizowano te zależności u dzieci w stanie przedpokwitaniowym w stanie eutyreozy z idiopatycznym niskim wzrostem (ISS). Analizie poddano stężenie greliny oraz GH w testach stymulujących i w nocy oraz IGF-I, TSH, FT_4 i wolnej trójjodotyroniny (FT_3) u 85 dzieci z ISS (36 dziewczynek, 49 chłopców) w wieku 9,65 lat ±3,02 lata (średnia ±SD). Potwierdzono silną dodatnią korelację między greliną a TSH ($r = +0,44$, $p < 0,05$). Ponadto u dzieci z wyższym prawidłowym stężeniem TSH obserwowano wyższe stężenie greliny, ale niższe nocne wartości GH i niższy IGF-I w porównaniu do grupy dzieci z niższym prawidłowym TSH. Co ciekawe, zmiany stężenia TSH nie miały wpływu na stężenia FT_4 i FT_3. Podsumowując, u dzieci w okresie przedpokwitaniowym w stanie eutyreozy ISS wydzielanie greliny i TSH jest ściśle powiązane. Z drugiej strony, im wyższy TSH, tym niższe nocne poziomy GH i IGF-I. Powyższe ustalenia mogą być wstępem do dalszych badań nad rolą greliny i TSH w procesie wzrastania.

Publikacja Nr 3.

Adamczewska K, Adamczewski Z, Lewiński A, Stawerska R.

Leptin Does Not Influence TSH Levels in Obese Short Children.

Front Endocrinol (Lausanne). 2022 Mar 24;13:838881.

Hormon wzrostu (GH) i hormony tarczycy są ważne dla rosnących dzieci. U niektórych otyłych dzieci obserwuje się nieznacznie podwyższone stężenie TSH. Może to być mechanizm adaptacyjny: stymulacja biosyntezy pro-TRH w podwzgórzu w odpowiedzi na podwyższony poziom leptyny. Podwyższony TSH może również odzwierciedlać konieczność utrzymania spoczynkowego wydatku energetycznego lub może być wynikiem niewłaściwego, niskiego stężenia FT_4. W związku z tym oceniliśmy stężenie TSH i FT_4 w surowicy u dzieci z idiopatycznym niskim wzrostem (ISS) (bez niedoboru GH) oraz zbadaliśmy wpływ stanu

odżywienia dzieci i poziomów wybranych adipocytokin na czynność tarczycy, poszukując obecności różnych postaci subklinicznej niedoczynności tarczycy, co może być przyczyną spowolnienia tempa wzrostu. Grupę badaną stanowiło 115 dzieci (50 dziewcząt i 65 chłopców) z ISS w wieku (średnia ±SD) 10,4 ±3,34 lat. U każdego dziecka podczas testów stymulacyjnych oznaczono lipidogram, stężenia TSH, FT_4, IGF-1, maxGH, leptyny, adiponektyny i rezystyny. Na podstawie BMI SDS wyróżniono 3 podgrupy: szczupłą (n=26), otyłą (n=21) i prawidłową (n=68). Nie stwierdzono korelacji między poziomem leptyny a poziomami TSH, FT_4. Stężenia leptyny, cholesterolu całkowitego i LDL-cholesterolu u otyłych dzieci z niskim poziomem były istotnie wyższe niż u dzieci z innych podgrup. Z kolei poziomy adiponektyny, rezystyny, TSH i FT_4 nie różniły się między podgrupami. U 7% dzieci stwierdzono podwyższony poziom TSH (ale poniżej 10 mIU/L), z podobną częstością w podgrupach. Im wyższa leptyna, tym niższy maxGH w teście stymulacji klonidyną. Na podstawie powyższych obserwacji wysnuto wniosek, że u otyłych dzieci z idiopatycznym niskim wzrostem leptyna nie zwiększa wydzielania TSH. Może to być związane z zaburzeniem wpływu leptyny na produkcję TSH u tej grupy pacjentów i może wskazywać na szeroko zakrojone zaburzenia sygnałów z podwzgórza, a w konsekwencji być przyczyną nieprawidłowego wydzielania GH.

3. SUMMARY

The Influence of Non-Thyroid Factors on the Modulation of TSH Secretion in Children, Taking Into Particular Account Infectious Factors, Orexigenic Pathway Hormones and Hormones of the Adipose Tissue

Publication No 1.

Adamczewska K, Adamczewski Z, Stasiak M, Lewiński A, Stawerska R.

Transient Hyperthyrotropinemia in Outpatient Children with Acute Infections of the Respiratory System.

Int J Environ Res Public Health. 2021 Apr 13;18(8):4115.

Diagnostics of thyroid disorders (TD) are frequently based on the measurements of thyroid stimulating hormone (TSH) concentration only. If TSH is outside the reference range, the diagnostic procedure used in patients with TD is introduced. Observations indicate that in a considerable number of these patients, TD is not confirmed. The aim of the study was to assess the incidence of transient hyperthyrotropinemia in healthy children during acute infections of the respiratory system. Patients and Methods: The study included consecutive children (49 boys and 45 girls), aged 2.2–17.3 years, who visited one General Practitioner (GP) due to respiratory tract infections. The tests: complete blood count (CBC), C-reactive protein (CRP), TSH and free thyroxine (FT_4) were run on the next day after the visit at the physician's (initial visit) and ≥2 weeks after recovery. Results: Among these children, elevated TSH values were found in about 10% of patients, and they went back to normal values after recovery. A prospective analysis showed a reduction of TSH values in approx. 65% of all groups and TSH at the follow-up visit was significantly lower. Conclusions: Transient hyperthyrotropinemia was observed in about 10% of children with acute respiratory tract infection. This allowed to draw a clinically significant conclusion that in the case of a moderately elevated TSH value in children in the course of respiratory tract infection, it is necessary to perform a control test after recovery.

Publication No 2.

Adamczewska K, Adamczewski Z, Łupińska A, Lewiński A, Stawerska R.

Strong Positive Correlation between TSH and Ghrelin in Euthyroid Non-Growth Hormone-Deficient Children with Short Stature. Molecules. 2020 Aug 27;25(17):3912.

The growth processes in children depend on the proper functioning of some hormones and growth factors. Recently, a positive correlation between ghrelin and TSH (thyroid stimulating hormone) in patients with hyper- and hypothyroidism was proved. Moreover, in hypothyroid rats with high ghrelin concentration, growth hormone (GH) and insulin-like growth factor I (IGF-I) secretion was suppressed. We analyzed these relationships in euthyroid prepubertal children with idiopathic short stature (ISS). The analysis comprised concentration of ghrelin, GH in stimulating tests and during the night, as well as IGF-I, TSH, FT_4 and free triiodothyronine (FT_3) in 85 children with ISS (36 girls, 49 boys) aged 9.65 ±3.02 years (mean ±SD). A strong positive correlation between ghrelin and TSH was confirmed ($r = +0.44$, $p < 0.05$). A higher ghrelin but lower nocturnal GH and lower IGF-I were observed in children with higher normal TSH concentration than those in children with lower normal TSH. Interestingly, alterations of TSH level were without any impact on FT_4 and FT_3 concentrations. Summing up, in ISS prepubertal euthyroid children, ghrelin and TSH secretion are closely related. On the other hand, the higher the TSH, the lower the nocturnal GH and IGF-I levels. The contribution of the above findings in deterioration of growth processes requires further studies.

Publication No 3.

Adamczewska K, Adamczewski Z, Lewiński A, Stawerska R.

Leptin Does Not Influence TSH Levels in Obese Short Children.

Front Endocrinol (Lausanne). 2022 Mar 24;13:838881.

Growth hormone (GH) and thyroid hormones are important for children growing. In some obese children a slightly elevated TSH concentration is observed. This may be an adaptive mechanism: stimulation of pro-TRH biosynthesis in the hypothalamus in response to elevated leptin. The increased TSH may also reflect the necessity of maintaining the resting energy expenditure or may be a result of inappropriate, low FT_4 concentration. Thus, we evaluated serum TSH and FT_4 concentrations in idiopathic short stature (ISS) children (non GH-deficient) and examined the effect of children's nutritional status and levels of selected adipocytokines on thyroid function, searching for the presence of various forms of subclinical

hypothyroidism, which may be the cause of the slow growth rate. The study group included 115 children (50 girls and 65 boys) with ISS, aged (mean ±SD) 10.4 ±3.34 years. In each child, lipids, TSH, FT_4, IGF-1, maxGH during the stimulation tests, leptin, adiponectin and resistin concentrations were determined. Based on BMI SDS, 3 subgroups: slim (n=26), obese (n=21) and normal weight (n=68) were distinguished. There was no correlation between leptin level and TSH, FT_4 levels. The levels of leptin, total cholesterol and LDL-cholesterol in obese short children were significantly higher than in children from other subgroups. In turn, the levels of adiponectin, resistin, TSH and FT_4 did not differ between subgroups. In 7% of children, an elevated TSH level was found (but less than 10 mIU/L), with a similar frequency across subgroups. The higher the leptin, the lower maxGH in clonidine stimulation test was recorded. It seems that in obese children with idiopathic short stature leptin does not increase TSH secretion. This may be related to a disruption of the effect of leptin on TSH production and could indicate wide ranging disturbances of hypothalamic signals, and consequently be the cause of inappropriate GH secretion.

4. Komentarz do cyklu publikacji w języku polskim

„Wpływ czynników pozatarczycowych na modulację wydzielania TSH u dzieci ze szczególnym uwzględnieniem czynników infekcyjnych, hormonów szlaku oreksygenicznego i hormonów tkanki tłuszczowej"

Hormon tyreotropowy (TSH) jest istotnym elementem osi podwzgórze-przysadka-tarczyca, a oznaczanie jego stężenia w surowicy krwi stanowi podstawę do wnioskowania w diagnostyce laboratoryjnej dotyczącej funkcji tarczycy. Sprzężenie zwrotne ujemne pomiędzy hormonami wytwarzanymi w tarczycy (FT_4 i FT_3) a wydzielaniem hormonu uwalniającego TSH (TRH) z podwzgórza i TSH z przysadki jest podstawowym mechanizmem regulującym stan tyreometaboliczny człowieka. Nie zawsze jednak nieprawidłowe stężenie TSH świadczy o zaburzeniach czynności gruczołu tarczowego; istnieje bowiem wiele innych czynników, które wpływają na syntezę i wydzielanie TSH. Oparcie oceny stanu tyreometabolicznego o izolowane oznaczenie tego hormonu może zatem prowadzić do postawienia fałszywego rozpoznania. Interpretacja wyniku badania TSH nie może być oderwana od kontekstu klinicznego i od oceny wpływu pozatarczycowych czynników, które mogą wpływać na jego stężenie i prowadzić do podejmowania niewłaściwych decyzji diagnostyczno-terapeutycznych.

Na rozprawę doktorską składa się cykl 3 spójnych tematycznie prac, w których oceniano wpływ czynników pozatarczycowych na modulację wydzielania TSH u dzieci ze szczególnym uwzględnieniem czynników infekcyjnych, wybranych hormonów szlaku oreksygenicznego i hormonów tkanki tłuszczowej.

Publikacja Nr 1.

Adamczewska K, Adamczewski Z, Stasiak M, Lewiński A, Stawerska R.

Transient Hyperthyrotropinemia in Outpatient Children with Acute Infections of the Respiratory System.

Int J Environ Res Public Health. 2021 Apr 13;18(8):4115.

Istnieją doniesienia wskazujące na zmiany wydzielania TSH w wyniku toczących się procesów zapalnych. W praktyce klinicznej obserwowane jest podwyższone stężenie TSH u dzieci w trakcie infekcji dróg oddechowych. Niejednokrotnie stwierdzano w takich przypadkach umiarkowanie podwyższone stężenie tego hormonu. W wielu przypadkach

stanowiło to podstawę rozpoznania/podejrzenia niedoczynności tarczycy i było przyczyną wdrażania specjalistycznej diagnostyki w ramach poradni endokrynologicznej, która nie potwierdzała choroby tarczycy. Aby uniknąć takich sytuacji podjęto próbę oceny czy występuje istotna statystycznie różnica między stężeniem TSH oznaczonym w trakcie infekcji a tym oznaczonym w pełni zdrowia.

Celem pracy była analiza wpływu ostrej infekcji dróg oddechowych na stężenie TSH u dzieci.

Do badania zostały zakwalifikowane dzieci zgłaszające się do POZ z powodu ostrej infekcji dróg oddechowych.

Przebadano łącznie 94 dzieci (49 chłopców i 45 dziewcząt) w wieku od 2.2 do 17.3 lat. Z badań wyłączono dzieci z wcześniej stwierdzonymi chorobami tarczycy. Oceniano stan ogólny dzieci, występowanie lub brak gorączki, oznaczano morfologię krwi obwodowej ze wzorem odsetkowym leukocytów, białko C-reaktywne oraz dwukrotnie stężenie TSH i FT4: następnego dnia po zgłoszeniu się do poradni oraz po około 2 tygodniach po wyzdrowieniu.

Wśród badanych dzieci u około 10% stwierdzono umiarkowanie podwyższone stężenie TSH (przy prawidłowym stężeniu FT_4), które uległo normalizacji po powrocie do zdrowia. Zaobserwowano również, że stężenia TSH u około 65% dzieci w trakcie infekcji (pozostając w zakresach wartości referencyjnych) były istotnie wyższe w stosunku do badania kontrolnego. Przyczyny tego zjawiska pozostają niejasne, lecz efektem pracy jest bardzo istotne klinicznie spostrzeżenie o konieczności wykonania kontrolnego badania stężenia TSH w przypadku stwierdzenia umiarkowanie podwyższonej wartości tego hormonu w trakcie trwającej ostrej infekcji układu oddechowego, co pozwoli unikać kosztownej i uciążliwej dla dziecka diagnostyki w poradni specjalistycznej.

Publikacja Nr 2.

Adamczewska K, Adamczewski Z, Łupińska A, Lewiński A, Stawerska R.

Strong Positive Correlation between TSH and Ghrelin in Euthyroid Non-Growth Hormone-Deficient Children with Short Stature. Molecules. 2020 Aug 27;25(17):3912.

Jak wspomniano, TSH jest uwalniany z przysadki w odpowiedzi na stymulacyjne działanie TRH, który produkowany jest w podwzgórzu. Oprócz oczywistego sprzężenia zwrotnego ujemnego jakie zachodzi dla tej osi (hamowanie wydzielania TRH i TSH w odpowiedzi na podwyższone stężenie hormonów tarczycy: FT_4 i FT_3), wiele innych czynników wpływa modulująco na wydzielanie hormonów podwzgórzowych i przysadkowych. Jednym z nich

jest odkryta stosunkowo niedawno grelina. Hormon ten jest produkowany w żołądku pod wpływem głodu. Stymuluje szlak oreksygeniczny w podwzgórzu, co w efekcie powoduje chęć przyjęcia posiłku. Z drugiej strony grelina jest
jednym z najsilniejszych stymulatorów wydzielania hormonu wzrostu (GH), a przypuszcza się, że stymuluje produkcję również innych hormonów przysadki. Ostatnio dowiedziono, że u osób z niedoczynnością tarczycy stężenia greliny są podwyższone, zaś u osób z nadczynnością tarczycy – obniżone. Wykryto również silną dodatnią korelację pomiędzy stężeniem greliny a stężeniem TSH u pacjentów z nieprawidłową czynnością tarczycy (zarówno z hipo- jak i z hipertyreozą).

Ponieważ wiadomo, że proces wzrastania u dzieci jest uwarunkowany prawidłowym działaniem GH i hormonów tarczycy, niezwykle ciekawym zagadnieniem jest ustalenie wpływu jaki wywiera grelina na osie: GH/insulinopodobny czynnik wzrostu (IGF-1) oraz na TSH/hormony tarczycy, zwłaszcza w odniesieniu do niskorosłych dzieci, u których nie stwierdzono niedoboru GH ani niedoczynności tarczycy.

Celem pracy była analiza wzajemnych korelacji pomiędzy wydzielaniem greliny, GH, IGF-1 oraz TSH i FT_4 u dzieci z eutyreozą (stężenie TSH i FT_4 w zakresie normy) i idiopatycznym niedoborem wzrostu (ISS), tj. nie związanym z niedoborem GH ani żadną inną znaną przyczyną niedoboru wzrostu.

Badaniem objęto 85 dzieci (36 dziewcząt i 49 chłopców) w wieku średnio 9.65 +/- 3.02 lat. Wykazano silną dodatnią zależność między stężeniem greliny a stężeniem TSH ($r = +0.44$, $p<0.05$). W grupie dzieci, u których stężenie TSH znajdowało się w górnym przedziale zakresu normy (stężenie równe lub wyższe niż mediana czyli 2.29 mIU/ml) stwierdzono wyższe stężenie greliny i jednocześnie niższe nocne wydzielanie GH oraz niższe stężenie IGF-1 w porównaniu do dzieci z TSH poniżej 2.29 mIU/ml.

Na podstawie powyższych obserwacji stwierdzono, że u dzieci z idiopatycznym niedoborem wzrostu istnieje silna dodatnia korelacja pomiędzy wydzielaniem greliny i TSH. Dodatkowo, niezwykle interesującym spostrzeżeniem jest, że im wyższe jest stężenie greliny i TSH, tym niższe nocne wydzielanie hormonu wzrostu i IGF-1. Trudno wytłumaczyć, dlaczego - pomimo wysokiego stężenia greliny - stężenie GH i IGF-I pozostaje obniżone. Można przypuszczać, iż raczej podwyższony poziom greliny (jak i być może TSH) jest odpowiedzią na obniżone stężenie IGF-1. Powyższa obserwacja jest wstępem do dalszych badań nad rolą greliny i TSH w procesie wzrastania.

Jednocześnie, w związku z brakiem bezpośredniego przełożenia stężenia TSH i/lub

greliny na stężenie FT_4, nie wydaje się, aby dzieci z ISS, u których stężenie FT_4 pozostaje w dolnym tercylu wartości prawidłowych lub u których stężenie TSH pozostaje w górnej połowie zakresu wartości prawidłowych wymagały leczenia L-tyroksyną.

Publikacja Nr 3.

Adamczewska K, Adamczewski Z, Lewiński A, Stawerska R.

Leptin Does Not Influence TSH Levels in Obese Short Children.

Front Endocrinol (Lausanne). 2022 Mar 24;13:838881.

U niektórych otyłych dzieci obserwuje się podwyższone stężenie TSH przy prawidłowym stężeniu FT_4. Istnieją co najmniej dwie możliwości wyjaśnienia tego stanu. Z jednej strony zwiększona produkcja TSH może być wyrazem mechanizmu adaptacyjnego, mającego na celu zwiększenie spoczynkowego wydatku energetycznego. Mechanizm ten jest wywołany, między innymi, zwiększonym stężeniem leptyny (jednego z hormonów tkanki tłuszczowej, jej stężenie w surowicy jest silnie dodatnio skorelowane z masą ciała), które bezpośrednio stymuluje produkcję pro-TRH w podwzgórzu, a ca za tym idzie wzmożoną produkcję TRH i TSH.

Z drugiej strony, podwyższone stężenie TSH może być wykładnikiem subklinicznej niedoczynności tarczycy (nieadekwatnie niskiego stężenia FT_4). Niedoczynność tarczycy jest - z kolei - znaną przyczyną wolnego tempa wzrastania i niskorosłości. Rozróżnienie tych dwóch stanów w przypadku niskiego, otyłego dziecka z umiarkowanie podwyższonym TSH jest zatem kluczowe. Poszukiwanie zależności pomiędzy hormonami wytwarzanymi przez adipocyty a hormonami podwzgórzowymi nadal jest przedmiotem badań naukowych.

Celem pracy nr 3 była ocena wpływu stanu odżywienia i stężeń wybranych hormonów tkanki tłuszczowej (adipocytokin) na stężenia TSH i FT_4 u dzieci z idiopatycznym niedoborem wzrostu (ISS), u których nie stwierdza się morfologicznych czy autoimmunizacyjnych wykładników choroby tarczycy. Badaniem objęto 115 dzieci (50 dziewcząt i 65 chłopców) z ISS w wieku średnio 10,4+/- 3,34 lat. U wszystkich dzieci oznaczano stężenie TSH, FT_4, leptyny, adiponektyny i rezystyny, IGF-1 oraz oceniono wydzielanie GH podczas testów stymulacyjnych. Na podstawie obliczonego wskaźnika masy ciała (BMI SDS), dzieci podzielono na 3 podgrupy: szczupłe (n=26), otyłe (n=21) i o prawidłowej masie ciała (n=68).

W grupie dzieci otyłych stwierdzono wyższe - niż w pozostałych podgrupach - stężenie leptyny, nie wykryto jednak spodziewanej dodatniej korelacji między stężeniem

leptyny a stężeniem TSH. Stężenie TSH i FT_4 w tej grupie nie różniły się od stężeń tych hormonów w grupach dzieci szczupłych i o prawidłowej masie ciała. Umiarkowanie podwyższone stężenie TSH (nie przekraczające 10 mIU/ml) obserwowano u dzieci we wszystkich podgrupach z jednakową częstością, łącznie dotyczyło 7% dzieci. Wydaje się wobec tego, że u dzieci otyłych z ISS wyższe stężenie leptyny nie wpływa na zwiększenie wydzielania TSH.

Zaobserwowano również, że wyższym stężeniom leptyny towarzyszyło niższe (niż w pozostałych podgrupach) wydzielanie GH w teście stymulacyjnym po podaniu klonidyny. Brak stymulującego wpływu leptyny na sekrecję TSH i gorsze wydzielanie GH w tych przypadkach wskazuje zatem na możliwość zaburzeń przewodnictwa sygnałowego w podwzgórzu, dotyczącego zarówno TSH, jak i GH, co w konsekwencji być przyczyną nieprawidłowego tempa wzrastania. Stwierdzenie podwyższonego TSH u niskiego, otyłego dziecka wymaga zatem czujności i zindywidualizowanego podejścia diagnostyczno-terapeutycznego. Jest to również zagadnienie wymagające dalszych badań.

Wnioski uzyskane z powyższych prac potwierdzają postawioną na wstępie tezę, że interpretacja wyniku badania stężenia TSH w surowicy krwi u dzieci powinna być oparta o ocenę stanu klinicznego. Stężenie TSH, zawierające się w granicach wartości referencyjnych, nie jest dowodem na prawidłowe funkcjonowanie osi podwzgórze-przysadka-tarczyca jak wykazano w grupie niskorosłych otyłych dzieci. Z drugiej strony podwyższone stężenie TSH nie zawsze stanowi wskazanie do wdrożenia terapii preparatem L-tyroksyny.

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5. Komentarz do cyklu publikacji w języku angielskim

The Influence of Non-Thyroid Factors on the Modulation of TSH Secretion in Children, Taking Into Particular Account Infectious Factors, Orexigenic Pathway Hormones and Hormones of the Adipose Tissue.

The thyreotropic hormone (TSH) is an important element of the hypothalamus-pituitary-thyroid gland (HPT) axis. Determining its serum concentration is necessary for drawing conclusions regarding the function of the thyroid gland in laboratory diagnostics. The negative feedback loop between the hormones produced in the thyroid gland (FT_3, FT_4), the secretion of the thyrotropin-releasing hormone (TRH) from the hypothalamus gland, and secretion of TSH from the pituitary gland is the basic regulatory mechanism of the human thyreometabolic state. However, abnormal TSH concentration does not always indicate the dysfunction of the thyroid gland; there are many other factors that influence the synthesis and secretion of TSH. Therefore, the evaluation of thyreometabolic state, based exclusively on the determination of this hormone may lead to false results. The interpretation of the TSH test can not be removed from the clinical context and the impact of non-thyroid factors, as these components might affect its concentration, and in turn lead to incorrect diagnostic and therapeutic decisions.

The doctoral dissertation consists of a series of 3 original manuscripts, thematically cohesive, assessing the modulation of TSH secretion, including the impact of non-thyroid factors, such as infectious factors, hormones of the orexygenic pathway and adipose tissue hormones.

Publication No 1

Adamczewska K, Adamczewski Z, Stasiak M, Lewiński A, Stawerska R.

Transient Hyperthyrotropinemia in Outpatient Children with Acute Infections of the Respiratory System.

Int J Environ Res Public Health. 2021 Apr 13;18(8):4115.

There are reports indicating changes in TSH secretion as a result of ongoing inflammatory processes. In clinical practice, moderately elevated concentration of TSH in children with common respiratory tract infections is observed. In many cases, it is the basis for the diagnosis or suspicion of hypothyroidism, and is the reason for the implementation of expensive and time-consuming diagnostics in endocrine clinics. Clinical observations indicate

that in a considerable number of this cases, thyroid disease is not confirmed, and the result of the initial tests are - in fact - falsely positive. The study attempted to asses whether there is a statistically significant difference between the TSH concentration determined during infection and that determined in full health.

The aim of the study was to analyze the influence of acute respiratory tract infection on TSH concentration in children.

The study included children who visited a single Primary Health Centre due to acute respiratory tract infections. A total 94 children were examined (49 boys and 45 girls) between the ages from 2,2 to 17,3. Children with previously diagnosed thyroid diseases were excluded from the study. The general condition of children, the presence or absence of fever were assessed. The tests: complete blood count (CBC), C-reactive protein (CRP), TSH and FT4 were run on the next day after visit at the physician's (initial visit) and about 2 weeks after recovery.

Among these children, elevated TSH values were found in about 10% of children. At the same time FT4 concentration was in normal range. TSH went back to the normal values after recovery. It was also observed that the concentration of TSH in about 65% of children during the infection was significantly elevated compared to the control study, but remained within the ranges of the reference values. The etiology of this finding is unclear, but our study demonstrated that acute infection is a condition that may lead to transient elevation of TSH which is a clinically relevant observation, and if the test is performed during an infection and a slightly elevated TSH concentration is noted, the TSH test should be repeated after recovery. Such an approach will allow many patients to avoid unnecessary diagnostic procedures and implementation of treatment.

Publication No 2.

Adamczewska K, Adamczewski Z, Łupińska A, Lewiński A, Stawerska R.

Strong Positive Correlation between TSH and Ghrelin in Euthyroid Non-Growth Hormone-Deficient Children with Short Stature. Molecules. 2020 Aug 27;25(17):3912.

As mentioned, TSH is released from the pituitary in response to the stimulating effects of TRH, which is produced in the hypothalamus. Apart from the obvious negative feedback on this axis (inhibition of TRH and TSH secretion in response to elevated levels of thyroid hormones: FT4 and FT3), many other factors modulate the secretion of hypothalamic and pituitary hormones. One of them is ghrelin, discovered relatively recently. This hormone is

produced in the stomach, and its production is influenced by starvation. It stimulates the orexigenic pathway in the hypothalamus, which results in the desire to eat. Other than that,

ghrelin is one of the strongest growth hormone (GH) secretagogues, and is believed to stimulate the production of other pituitary hormones as well. Recently, it has been shown that people with hypothyroidism have increased ghrelin levels, and people with hyperthyroidism - lowered ghrelin levels. A strong positive correlation was also found between ghrelin levels and TSH levels in patients with abnormal thyroid function (both with hypo- and hyperthyroidism).

As it is known that the growth process in children is conditioned by the proper functioning of GH and thyroid hormones, it is extremely interesting to determine the influence of ghrelin on the GH / insulin-like growth factor (IGF-1) axes and on TSH / thyroid hormones, especially in relation to short-grown children without GH deficiency or hypothyroidism.

The aim of the study was to analyze the interrelationships between the secretion of ghrelin, GH, IGF-1 as well as TSH and FT_4 in children with euthyreosis (TSH and FT_4 levels within the normal range) and idiopathic growth deficiency (ISS), i.e. not related to GH deficiency or any other known cause of growth deficiency.

The study included 85 children (36 girls and 49 boys) aged 9.65 +/- 3.02 years on average. A strong positive correlation was demonstrated between ghrelin concentration and TSH concentration ($r = +0.44$, $p < 0.05$). In the group of children with TSH concentration in the upper range of the normal range (concentration equal to or higher than the median, i.e. 2.29 μIU / ml), a higher ghrelin concentration and lower GH secretion at night and a lower concentration of IGF-1 were found compared to children with TSH concentration less than 2.29 μIU / ml.

Based on the above observations, it was found that in children with idiopathic growth deficiency there is a strong positive correlation between ghrelin secretion and TSH. Additionally, an extremely interesting finding is that the higher the concentration of ghrelin and TSH, the lower the nocturnal secretion of growth hormone and IGF-1. It is difficult to explain why - despite the high concentration of ghrelin - the concentration of GH and IGF-I remains reduced. It can be assumed that a rather increased level of ghrelin (and possibly TSH) is a response to a decreased concentration of IGF-1. The above observation is an introduction to further research on the role of ghrelin and TSH in the growth process.

At the same time, due to the lack of a direct correlation of TSH and / or ghrelin levels on FT_4 levels, it does not seem that children with ISS with FT4 levels in the lowest tercyle of the normal range, as well as ISS children with TSH level in the upper normal range (above the median value), do not require treatment with L-T4.

Publication No 3.

Adamczewska K, Adamczewski Z, Lewiński A, Stawerska R.

Leptin Does Not Influence TSH Levels in Obese Short Children.

Front Endocrinol (Lausanne). 2022 Mar 24;13:838881.

Some obese children have elevated TSH levels with normal FT_4 levels. There are at least two possible explanations for this condition. The increased TSH production may be an expression of an adaptive mechanism aimed at increasing resting energy expenditure. This mechanism is caused, inter alia, by the increased concentration of leptin (one of the adipose tissue hormones, its serum concentration is strongly positively correlated with body weight), which directly stimulates the production of pro-TRH in the hypothalamus, and thus the increased production of TRH and TSH.

On the other hand, elevated TSH levels may be indicative of subclinical hypothyroidism (inadequately low FT_4 levels). Hypothyroidism is, in turn, a known cause of slow growth and short stature. The distinction between the two conditions in a short, obese child with moderately elevated TSH is therefore crucial. The relationship between hormones produced by adipocytes and hypothalamic hormones is still the subject of scientific research.

The aim of study no. 3 was to evaluate the effects of nutritional status and concentrations of selected adipose tissue hormones (adipocytokines) on TSH and FT_4 levels in children with idiopathic growth deficiency (ISS), who do not have morphological or autoimmune thyroid disease markers. The study included 115 children (50 girls and 65 boys) with ISS, mean age 10.4 +/- 3.34 years. The concentration of TSH, FT_4, leptin, adiponectin and resistin, IGF-1 was measured in all children and GH secretion was assessed during stimulation tests. Based on the calculated body mass index (BMI SDS), children were divided into 3 subgroups: slim (n = 26), obese (n = 21) and normal weight (n = 68).

In the group of obese children, the concentration of leptin was higher than in the remaining subgroups, but the expected positive correlation between the concentration of leptin and the concentration of TSH was not detected. The levels of TSH and FT4 in this group did not

differ from the levels of these hormones in the groups of slim and healthy children. Moderately elevated TSH levels (not exceeding 10 μIU / ml) were observed in children in all subgroups with the same frequency, in a total of 7% of children. It therefore seems that in obese children with ISS, higher leptin levels do not increase TSH secretion.

It was also observed that higher leptin concentrations were associated with lower (than in other subgroups) GH secretion in the stimulation test after administration of clonidine. The lack of a stimulating effect of leptin on TSH secretion, and lesser GH secretion in these cases therefore indicate the possibility of disturbances in signal conduction in the hypothalamus concerning both TSH and GH, which in turn may be the cause of abnormal growth rate. Therefore, the diagnosis of an increased TSH in a short, obese child requires vigilance and an individualized diagnostic and therapeutic approach. It is also an issue that requires further research.

Conclusions obtained from these manuscripts confirm the introductory thesis that the interpretation of the concentration of TSH in blood serum in children should not be removed from the patients' clinical presentation. TSH concentration in the normal range is not synonymous with correct functioning of the hypothalamus-pituitary-thyroid gland axis, as proved in the group of children with short stature. On the other hand, elevated concentration of TSH is not always an indication for implementation of treatment with L-thyroxine.

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6. Kopie prac tworzących cykl publikacji

Rozdziały 6.1-6.3 stanowią odrębne publikacje z osobną bibliografią na zakończenie każdej z nich.

6.1 Transient Hyperthyrotropinemia in Outpatient Children with Acute Infections of the Respiratory System

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Article

Transient Hyperthyrotropinemia in Outpatient Children with Acute Infections of the Respiratory System

Katarzyna Adamczewska [1], Zbigniew Adamczewski [2,3], Magdalena Stasiak [3], Andrzej Lewiński [3,4] and Renata Stawerska [3,5,*]

1 Primary Care Unit "Medyk"–Outpatient Clinic, 95-035 Ozorków, Poland; kadamczewska@o2.pl
2 Department of Nuclear Medicine, Medical University of Lodz, 92-216 Lodz, Poland; zbigniew.adamczewski@umed.lodz.pl
3 Department of Endocrinology and Metabolic Diseases, Polish Mother's Memorial Hospital-Research Institute, 93-338 Lodz, Poland; mstasiak33@gmail.com (M.S.); andrzej.lewinski@umed.lodz.pl (A.L.)
4 Department of Endocrinology and Metabolic Diseases, Medical University of Lodz, 93-338 Lodz, Poland
5 Department of Pediatric Endocrinology, Medical University of Lodz, 93-338 Lodz, Poland
* Correspondence: renata.stawerska@umed.lodz.pl; Tel.: +48-42-2711141

Abstract: Background: Diagnostics of thyroid disorders (TD) are frequently based on the measurements of thyroid stimulating hormone (TSH) concentration only. If TSH is outside the reference range, the diagnostic procedure used in patients with TD isintroduced. Observations indicate that in a considerable number of these patients, TD is not confirmed. The aim of the study was to assess the incidence of transient hyperthyrotropinemia in healthy children during acute infections of the respiratory system. Patients and Methods: The study included consecutive children (49 boys and 45 girls), aged 2.2–17.3 years, who visited one General Practitioner (GP) due to respiratory tract infections. The tests: complete blood count (CBC), C-reactive protein (CRP), TSH and FT4 were run on the next day after the visit at the physician's (initial visit) and ≥2 weeks after recovery. Results: Among these children, elevated TSH values were found in about 10% of patients, and they went back to normal values after recovery. A prospective analysis showed a reduction of TSH values in approx. 65% of all groups and TSH at the follow-up visit was significantly lower. Conclusions: Transient hyperthyrotropinemia was observed in about 10% of children with acute respiratory tract infection. This preliminary finding remains unexplained.

Keywords: thyroid stimulating hormone; transient hyperthyrotropinemia; acute respiratory tract infection; children

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1. Introduction

Normal thyroid hormone (thyroxine-T4 and triiodothyronine-T3) secretion and action are essential for both fetal and post-natal neurodevelopment. It is also important for, normal growth and metabolic processes in children [1,2]. The prevalence of thyroid disease in Polish children is not very well known, but in the first two decades after mandatory salt iodination, we observed a lower incidence of goiter and a higher frequency of Graves' disease [3,4]. In the iodine-sufficient regions, the most common cause of acquired primary hypothyroidism (characterized by low free T4 (FT4) and elevated thyroid stimulating hormone (TSH) serum concentrations) is autoimmune hypothyroidism (Hashimoto's thyroiditis); its prevalence in childhood has been estimated as 1–2% [1].

In turn, in approximately 2% of children, subclinical hypothyroidism is present. It can be defined as primary biochemically compensated hypothyroidism [1,2]. In these conditions, normal FT4 and free T3 (FT3) and elevated TSH serum concentrations are observed. The treatment of subclinical hypothyroidism is controversial, as it is usually an asymptomatic, benign condition with rare progression to over hypothyroidism. According to the current recommendations, only symptomatic children, children younger than 3

years old, or those with TSH levels higher than 10 mIU/L require the L-T4 treatment [5–7]. The etiology of subclinical hypothyroidism is usually idiopathic–especially in the cases of transient hyperthyrotropinemia, but it also may be the initial manifestation of Hashimoto's thyroiditis [5,6,8].

About 50% of patients with childhood autoimmune hypothyroidism have a family history of such diseases [1]. It is well known that the occurrence of autoimmune thyroid disease (AITD) depends onan interaction between genetic susceptibility and environmental factors. Thus, there is an ongoing debate in scientific associations andthe general public about the factors that can initiate hypothyroidism. As a result, parents or legal guardians often ask General Practitioners (GPs) to screen their children for thyroid diseases.

Diagnostics of thyroid dysfunctions arefrequently based on the measurements of TSH serum concentration only. This is due to the fact that alog-linear relationship between TSH and FT4 and FT3 concentration is observed, thus abnormal TSH concentration suggests the possible occurrence of thyroid disorders [9,10]. In children, the level of TSH appears to be a stable and solid diagnostic parameter, however, its variability in laboratory tests has been observed [11]. On the other hand, there are some conditions in which the transitionally elevated TSH concentration is observed, with obesity being one of the well-documented examples [12]. Studies concerning the influence of other transient conditions (e.g., inflammations) on TSH secretion are divergent [13,14]. However, it was proven that the elevation of several proinflammatory cytokines may affect thyroid function tests, which mimic thyroid disorders in the absence of actual thyroid disease [15,16]. The best-known example of changes in the thyroid axis function, occurring also in children, is a non-thyroidal illness syndrome (NTIS) in critically ill patients. This phenomenon can resemble a response of healthy subjects to fasting [17]. Thyroid hormone inactivation with low T3 and high rT3 followed by suppressed TSH is the most frequently observed phenomenon in these patients. However, NTIS may also lead to elevated TSH. It was demonstrated that during the recovery phase from such diseases serum TSH level may be increased, but generally not higher than 10 mIU/L [8]. The slightly elevated TSH levels after acute infections were observed in adults [18]. The influence of NTIS on TSH concentration in children is well documented. In turn, TSH changes during the most common infections of the respiratory tract in children are observed in practice, however, data on their incidence and causes are scarce. This phenomenon was found because of the tendency to combine TSH tests with diagnostic tests made due to other reasons (e.g., infection), in order to minimize stressful situations for a child. If TSH serum concentration is outside the reference range, the diagnostic procedure used in patients with thyroid disorders isintroduced. Clinical observations indicate that in a considerable number of these patients, thyroid disease is not confirmed, and the results of the initial tests are–in fact–falsely positive.

Thus, the aim of the study was to assess the incidence of transient hyperthyrotropinemia in generally well children during acute infections of the respiratory system.

2. Patients and Methods

The research was approved by the Bioethical Committee at the Polish Mother's Memorial Hospital Research Institute (PMMH-RI) in Lodz (approval code 69/2018).

The study included consecutive children who visited one GP (K.A.) in a single Primary Healthcare Centre due to mild or moderate respiratory tract infections over the period of one year. In every case, medical history, as well as signs and symptoms of the infection were recorded. Each child was physically examined, i.e., the throat and tonsils were assessed by inspection, the lungs were auscultated using a stethoscope and body temperature was checked using a non-contact thermometer. No chest X-rays were performed. Next, in every case, a complete blood count (CBC) test with differential was performed and C-reactive protein (CRP) in serum was measured. The tests were run on the next day after the visit at the physician's (peripheral blood samples were collected between 8.00 and 10.00 a.m., after overnight fasting). Venous blood was obtained by venipuncture (needle gauge 19). At the

same time, an additional blood sample was collected to assess TSH and FT4 concentrations. Moreover, during the visit, on the basis of the height and body mass measurements, the child's nutritional status was evaluated, by assessment of the body mass index (BMI) value. Obese (BMI > +2.0 SD; n = 7) and undernourished (BMI < −2.0 SD; n = 2) children were excluded from the study, similarly to patients treated for thyroid diseases (n = 2). At the time of data collection, none of the children displayed typical signs and symptoms of thyroid disorders, e.g., goiter.

On the basis of the medical history, physical examination and the results of laboratory tests, the type of infection was diagnosed, based on the ICD-10 classification. The children were qualified into one of the following diagnostic groups: A88.0-enteroviral exanthematous fever (Boston exanthem), H66.0-suppurative otitis media, J00-acute nasopharyngitis (common cold), J02-streptococcal pharyngitis, J03-acute tonsilitis, J04-acute laryngitis, J06-acute nasopharyngitis, J18-pneumonia (unspecified), J20-acute bronchitis, J36-peritonsillar abscess.

It was noted whether the infection was accompanied by a fever over 38 °C and elevated lab inflammatory markers (Table 1).

Table 1. The clinical characteristics of the analyzed group of patients.

ICD-10		No.	Fever >38 °C	Elevated CRP	Elevated WBC	Neutrophilia	Lymphocytosis	Elevated TSH
A88.0	Enteroviral exanthematous fever (Boston exanthema)	1	1	0	1	0	0	0
H66.0	Suppurative otitis media	2	2	2	1	2	2	2
J00	Acute nasopharyngitis (cold)	7	1	0	1	1	1	1
J02	Streptococcal pharyngitis	25	19	10	7	7	7	3
J03	Acute tonsilitis	2	2	0	0	0	0	0
J04	Acute laryngitis	11	0	3	2	4	4	1
J06	Acute nasopharyngitis	19	5	4	1	5	7	1
J18	Pneumonia (unspecified)	5	2	3	1	2	1	0
J20	Acute bronchitis	21	9	7	5	6	2	1
J36	Peritonsillar abscess	1	1	1	1	1	0	0
Total		94 (100%)	48 (51.1%)	30 (31.9%)	20 (23.4%)	28 (29.8%)	18 (19.1%)	9 (9.6%)

Next, the parents were asked to bring the child for a check-up examination after ≥2 weeks after recovery. At the follow-up visit, the patient's condition was evaluated again and a blood sample was taken between 8.00 and 10.00 a.m. in order to assess the same parameters: CBC, CRP, TSH and FT4. Thus, the blood samples were collected from the same patient at two consecutive time points: an initial visit during infection and a follow-up visit (which took place 2 weeks to 6 months after recovery). In each case where TSH was elevated the first time, the concentrations of thyroid peroxidase antibodies (a-TPO) and thyroglobulin antibodies (a-Tg) were assessed in the second blood sample.

Finally, 94 children (49 boys and 45 girls), aged 2.2–17.3 years, mean ±SD: 8.22 ± 3.98 years) were included in the study group. Among them, 76 children were qualified for the younger group: boys <12 years old and girls <11 years old, and 18 children for the older group: boys ≥12 years old and girls ≥11 years old.

The following parameters were considered significant inflammatory markers:

1. high CRP—according to World Health Organization recommendation-over 10 mg/L [19];
2. high white blood cell (WBC) count-above the reference range quoted on the test result, i.e., over 17.500 leukocytes/mm^3 for children up to 5 years old, over 15.000 leukocytes/mm^3 for children aged 6–12 years, and over 11.000 leukocytes/mm^3 for children over 12 years;
3. increase in the proportion of lymphocytes (lymphocytosis)-above the reference range quoted on the test result, i.e., over 60% for children up to 5 years old, over 48% for children aged 6–12 years, and over 45% for children over 12 years;
4. increase in the proportion of neutrophils (neutrophilia)-above the reference range quoted on the test result, i.e., 51% for children up to 5 years old, over 59% for children aged 6–12 years, and over 55% for children over 12 years.

Concentrations of TSH and FT4 were measured by the electrochemiluminescent immunoassays (ECLIA) method with commercially available appropriate kits (Roche Diagnostic, Mannheim, Germany). Normal range values were as follows: for TSH: age-dependent ranges-1–7 years-0.7–5.97 mIU/L; 7–12 years-0.6–4.84 mIU/L; 12–18 years-0.51–4.4 mIU/L with inter-assay coefficients of variation (CVs) 1.3–1.8% and for FT4: age-dependent ranges-1–6 years-0.96–1.77 ng/dL; 6–11 years-0.97–1.67 ng/dL; 11–18 years-0.98–1.63 ng/dL with CVs 2.0–2.4%. All assays were performed as a part of a standardpatient care in the same laboratory cooperating with the GP clinic. The range of WBC, TSH and FT4 reference values were established on the basis of data from this laboratory.

The data were analyzed using Statistica 11.0 PL software (StatSoft, Inc., Tulsa, OK, USA). The continuous variables were expressed as mean ±SD for normally distributed variables. Shapiro–Wilk test was used to test the distribution of the variables. The differences between girls and boys were compared using chi^2 test. Correlations were evaluated using the Pearson's test. A one-way ANOVA was applied for statistical analysis with the subsequent use of a post-hoc test, in order to statistically assess differences between groups; Tukey's test was selected because of the uneven amount of data in individual groups. To compare the frequency of cases with elevated TSH between the younger and the older group of children, Fisher's exact test was used. $p < 0.05$ was accepted as statistically significant value.

3. Results

In the study group of the 94 children, 68 (72.3%) were diagnosed with an infection of the upper respiratory tract, while 26 (27.7%)-with an infection of the lower respiratory tract. The infection was accompanied by fever in over half (51.1%) of the examined children. Elevated CRP was observed in 31.9%, neutrophilia or elevated WBC were found in 23.4% and 29.8%, respectively while lymphocytosis-in 19.1% of the patients. Table 1 presents the results of the analyzed study group depending on the clinical diagnosis.

In nine children from the studied group (9.6%), TSH concentration values exceeded the upper limit of the normal range, but none of them exceeded 7.0 mIU/L. In the check-up examination performed after recovery, elevated TSH values were not observed in any of the children (Figure 1). A detailed analysis of the cases in whichelevated TSH was found is presented in Table 2. Among children with elevated TSH found at the first time point (during infection), five patients had fever. In this subgroup (cases with elevated TSH concentration and fever), four children had elevated CRP, and among them, one had increased WBC and three neutrophilia, while in the fourth case, the CBC test result was normal. In the fifth child with fever, CRP and WBC were not elevated, but an increased number of neutrophils was observed. Three children did not have fever or elevation of any of the biochemical inflammatory markers.

Table 2. A detailed analysis of the cases where elevated thyroid stimulating hormone (TSH) was found.

					Initial Visit							Follow-Up Visit					
Gender	**Age (years)**	**Type of Infection**	**ICD-10**	**Fever**	**CRP mg/L**	**WBC 10^9/L**	**Neu %**	**Lym %**	**TSH mIU/L**	**FT4 ng/dL**	**Days between Initial and Follow-Up Visits**	**CRP mg/L**	**WBC 10^9/L**	**Neu %**	**Lym %**	**TSH mIU/L**	**FT4 ng/dL**
F	12	Upper resp. tract	H66	Yes	19.81 (↑)	13.9 (↑)	74.2 (↑)	13.9	4.57	1.33	30	<1	7.5	42	44.1	2.56	1.27
M	4	Upper resp. tract	H66	Yes	68.08 (↑)	10.1	52.5 (↑)	25.7	6.9	1.25	28	<1	8.06	37.2	49.8	4.02	1.19
M	11	Upper resp. tract	J00	No	<1	6.83	42.6	42.9	5.16	0.98	35	<1	6.44	50.1	36	3.04	1.21
F	13	Upper resp. tract	J02	Yes	7.03	5.34	59 (↑)	29	4.51	1.34	68	<1	4.13	49.9	39.7	2.91	1.29
F	14	Upper resp. tract	J02	Yes	10.51 (↑)	14.67(↑)	69.4(↑)	16.9	5.09	1.31	21	<1	6.85	43.9	45.0	4.36	1.23
M	6	Upper resp. tract	J02	No	3.26	11.33	32.1	55.5 (↑)	6.78	1.27	46	<1	5.77	56	33.8	5.77	1.33
F	3	Upper resp. tract	J04	No	<1	8.5	46.7	36.4	6.64	1.12	21	<1	7.91	29.2	58.3	5.56	1.18
F	11	Upper resp. tract	J06	No	1.04	9.3	44.9	43.9	5.95	1.22	37	<1	9.43	48	41.1	4.13	1.20
M	4	Lower resp. tract	J20	Yes	33.11 (↑)	4.21	35.9	35.9	5.85	1.44	15	<1	8.73	51	30.8	3.15	1.37

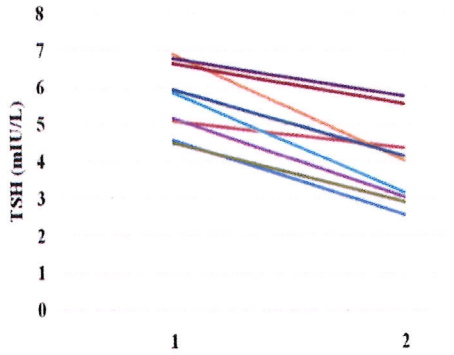

Figure 1. TSH concentration during infection (**1**) and during the check-up (**2**) in the group of children with elevated TSH concentration at the first examination (during infection).

In all of the analyzed cases, TSH concentration returned to normal in the check-up examination, performed after infection resolution. In none of the cases, FT4 concentration was reduced—the values ranged from 0.98 and 1.44 ng/dL, with the mean value of 1.25 ng/dL. An elevated a-TPO or a-Tg levels were not observed either.

In the whole group of the 94 examined children, TSH concentrations were analyzed with respect to the occurrence of inflammation symptoms mentioned above. Thus we compared TSH levels between the groups of children with and without fever, with normal and with elevated CRP, with normal and with elevated WBC, with normal and with an elevated count of neutrophils and with normal and with an elevated count of lymphocytes. Statistically significant differences between groups were not found, only in CRP-dependent analysis, the differences between groups were on the border of statistical significance (Table 3). A multivariance analysis confirmed that TSH level was not significantly influenced by any of the parameters in question (type of infection, fever, elevated value of CRP, WBC, elevated count of lymphocytes or neutrophils).

Table 3. The mean TSH levels (±SD) at the initial visit in children with and without fever, with and without elevated C-reactive protein (CRP), with and without elevated white blood cells (WBCs), with and without elevated neutrophils and with and without elevated lymphocytes.

	Normal	Elevated	p =
body temperature	2.93 ± 1.27	2.85 ± 1.27	0.76
CRP	2.74 ± 1.24	3.23 ± 1.27	0.08
WBC count	2.79 ± 1.18	3.23 ± 1.48	0.15
neutrophils count	2.83 ± 1.26	3.04 ± 1.27	0.48
lymphocytes count	2.90 ± 1.29	2.88 ± 1.23	0.98

We also compared TSH and FT4 levels between the younger (prepubertal) and older (pubertal) groups of children. We found no differences between the groups in terms of the parameters mentioned above. We found that elevated TSH was more common in older children, but the relevance of this finding is uncertain due to the unequal number of children in the two groups (Table 4).

Table 4. Characteristics of the analyzed group of children depending on the age (the younger group: boys <12 years old and girls <11 years old) and the older group: boys ≥12 years old and girls ≥11 years old).

	Younger Children n = 76	Older Children n = 18	*p* =
Girl/boys	32/44	13/5	
Age (years)	6.79 ±2.75	14.5 ±1.53	<0.000
TSH (mIU/L)	2.88 ± 1.29	2.95 ± 1.16	NS
FT4 (ng/mL)	1.24 ± 0.08	1.24 ± 0.11	NS
Elevated TSH n (%)	5 (6.6)	4 (22.2)	0.06

On the other hand, in the studied group of children, TSH concentration decreased in 61 out of 94 children (64.9%) in the check-up examination (after infection) regardless of its baseline value. Moreover, a reduction of more than 10% was observed in 37 children (39.4%). The mean values of TSH (±SD) were 2.93 ± 1.32 and 2.67 ± 1.05 mIU/L at the initial visit and the follow-up visit, respectively. The statistical analysis based on Wilcoxon's signed-rank test showed significant differences between TSH levels at the initial visit and the follow-up visit($p = 0.007$).

FT4 levels were within the normal range in all of the children and there was no significant correlation between TSH and FT4 levels, r = +0.10, $p > 0.05$(Figure 2). Moreover, FT4 concentration did not change between the initialand the follow-up visit.

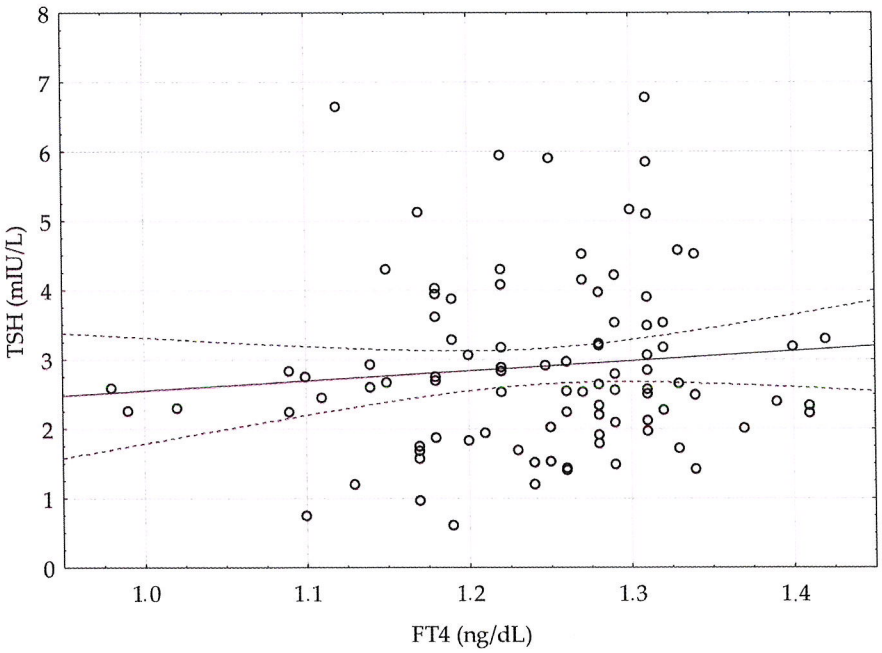

Figure 2. Correlation between TSH and FT4 concentration assessed in children during infection (Initial visit), r = +0.10, $p > 0.05$.

4. Discussions

Every disease, including infections, especially in the acute phase, disturbs the balance between anabolic and catabolic processes. Thyroid hormones play an important role in maintaining homeostasis in response to environmental challenges. Beneficial adaptation includes decreasing energy consumption and stimulation of an immunological response, which are crucial to survival [16].

The host's defense against infection involves the mechanisms of immunity and tolerance. While immunity promotes the elimination of pathogens, tolerance promotes adaptation to a given type of pathogen or to the intensity of the inflammatory response [20,21].

It is worth mentioning that there are two forms of adaptation activity. Type 1 allostasis occurs in diseases and conditions related to energy deficiency when the energy demand exceeds the supply and the stored energy resources. On the other hand, type 2 allostasis, occurs when the demand for energy does not exceed the potential supply, which is sufficient in relation to the needs [22,23]. Allostasis mediators involved in the acute phase reaction are proinflammatory cytokines. CRP is an acute-phase protein, whose synthesis in the liver is regulated by these cytokines. Its level is proportional to the concentration of inflammation mediators, which—in turn—positively correlates with the intensity of the inflammatory processes. CRP concentration exceeding 100 mg/L (often over 500 or 1000 mg/L) is usually found in serious bacterial infections. In the course of viral infections, usually normal or slightly elevated CRP concentrations are observed [24,25]. Therefore, we assessed CRP as one of the most important parameters indicating the type of infection and the phase response in our study.

The elevation of several proinflammatory cytokines may affect thyroid function tests. The acute phase of infection is mainly characterized by intensification of anterior pituitary hormone secretion and a peripheral inactivation of anabolic hormones [13]. One of the first alterations in acute illness is inhibition of type 1 deiodinase (D1) and type 2 deiodinase (D2) in peripheral tissues and subsequent impaired conversion of T4 to T3, leading to a decrease in serum and tissue T3 levels soon after the onset of acute illness [26]. D1 also deiodinates rT3, so the degradation is impaired and the levels of this inactive hormone rise simultaneously with the fall in T3 levels. Thus, TSH concentration temporarily increases in the first hours of the critical illness, which is followed by a temporary increase of T4 in the serum. Atthe same time, T3 concentration may already be reduced and reverse triiodothyronine (rT3) concentration may be elevated due to acute changes in the peripheral metabolism of thyroid hormone [27]. The scale of changes in T3 and rT3 concentration in the blood serum depends on the severity of the disease. This results in the NTIS [16]. In patients with mild to moderate NTIS, changes in T4 and TSH concentration are usually not observed, while patients suffering longer and from a more severe illness demonstrate low concentrations of T4 and TSH, as well [14].

In our study, in all the cases, we dealt with the acute form of common infection of the respiratory tract, which occurred in previously healthy, well-being children and was successfully cured in an outpatient clinic. Thus, the changes observed in NTIS, especially T3 and rT3 concentrations, were not a subject of our research. It is difficult to explain the reasons for the transient elevation of TSH levels observed in some children among our study group and the observed tendency to a higher TSH concentration during the infection period as compared to the period after recovery. We did not identify a significant correlation between increased TSH and individual, commonly available inflammatory markers, such as CRP, lymphocytosis and the presence of fever. Infection may trigger different reactions, depending on the kind of pathogen and the progress of infection. Thus, it may be difficult to clearly classify the observed abnormalities induces by infection into any of the two forms of adaptive activity. This may imply a more complex background of the phenomenon.

Temperature-induced changes of the thyroid or thyroid axis function are well known. One of them is the physiological increase in TSH concentration in response to exposure to cold, followed by an increase in the thyroid hormone synthesis, which stimulates metabolism [28].

However, the influence of high temperatures is ambiguous. The interesting data were provided by Oka et al. In their study, the evaluation of the influence of body temperature (BT), ranging from 37.5 to 40.5 °C, conducted in a group of 64 febrile patients, showed a relationship between BT and thyroid function. A negative correlation betweenTSH concentration and BT and a positive correlation between FT4 concentration and BT were observed. A detailed data analysis indicated that 8.2% of patients demonstrated an increased TSH concentration. This phenomenon was observed only for temperatures ranging from 37.5 to 38.5 °C and was not found in patients with higher temperatures [29].

Moreover, the experimental studies on the impact of temperature on thyroid function demonstrated that acute hyperthermia resulted in decreased blood flow to the thyroid gland and decreased secretion of FT4 and FT3, while TSH levels were not affected [30].

We also cannot disregard the significance of the loss of appetite during acute infection. The relationship between fasting and ghrelin, whose concentration correlates positively with TSH concentration, is well known. It is highly probable because the hormonal profile observed in the study group (increased TSH concentration during infection with no impact on FT4 concentration) is characteristic for the activity of ghrelin, whose elevated concentration directly inhibits T4 synthesis [31,32].

The highly complex processes, which take place in a living organism during acute infection may influence the thyroid axis at different levels and in an equally complex way. The concentrations of TSH and thyroid hormones are the result of all the processes that occur during the inflammatory reaction, which vary depending on the type and intensity of infection. Moreover, one of the key factors is the duration of infection, which is reflected in the severity and range of changes in the body functions. It should be remembered that even though changes in TSH concentration are usually not observed during NTIS, TSH transiently rises in the first hours of critical illness and during recovery [18,27].

Finding of TSH increase during mild or moderate upper respiratory tract infection may indirectly differentiate common infections in some children with coronavirus disease-19 (COVID-19), referring to the fact, the SARS-CoV-2 virus has a significant affinity to thyroid cells and may cause destructive thyroiditis and transient thyrotoxicosis accompanied by TSH suppression [33].

The limitations of our study, despite its prospective character, are differences in the time-lag between the infection onset and the exact moment when the patient first visited the GP and the patient's blood was drawn. Moreover, we did not determine the patients' hydration status with the application of the blood urea nitrogen/creatinine ratio assessment.

Our observation of the decreasing TSH concentrations after infection in nearly 2/3 of the children suggests that the secretion of this hormone during infection may result from simultaneous processes of stimulation (an increase in TSH concentration, typical of the first hours/days of infection, fasting, fever), as well as inhibition (D2 stimulation in the hypothalamus) of TSH secretion. The latter process occurs in parallel with the inhibition of the peripheral conversion of T4 to T3 [34].

Taking into account our results presented above, one should always remember that a diagnosis of thyroid dysfunctions made on the basis of a single TSH measurement only, though perhaps inexpensive and convenient, seems oversimplified and involves a considerable risk of obtaining falsely positive results. Our study demonstrated that acute infection is a condition that may lead to transient elevation of TSH, regardless of the level of inflammatory markers. Thus, the TSH level elevated in a child during or directly after an acute infection should be always re-assessed after the recovery.

5. Conclusions

1. Transient hyperthyrotropinemia was observed in about 10% of children suffering from an acute respiratory tract infection, regardless of its course, location and severity. The etiology for this finding is unclear.
2. If the test is performed during the infection and slightly elevated TSH concentration is noted, the TSH test should be repeated after recovery. Such an approach will allow many patients to avoid expensive diagnostic procedures and unnecessary implementation of treatment.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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6.2 Strong Positive Correlation between TSH and Ghrelin in Euthyroid Non-Growth Hormone-Deficient Children with Short Stature

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Strong Positive Correlation between TSH and Ghrelin in Euthyroid Non-Growth Hormone-Deficient Children with Short Stature

Katarzyna Adamczewska [1], Zbigniew Adamczewski [1,2], Anna Łupińska [1], Andrzej Lewiński [1,2] and Renata Stawerska [1,3,*]

1 Department of Endocrinology and Metabolic Diseases, Polish Mother's Memorial Hospital—Research Institute, 93-338 Lodz, Poland; kadamczewska@o2.pl (K.A.); zbigniew.adamczewski@umed.lodz.pl (Z.A.); ankalupinska@op.pl (A.Ł.); andrzej.lewinski@umed.lodz.pl (A.L.)

2 Department of Endocrinology and Metabolic Diseases, Medical University of Lodz, 93-338 Lodz, Poland

3 Department of Pediatric Endocrinology, Medical University of Lodz, 93-338 Lodz, Poland

* Correspondence: renata.stawerska@umed.lodz.pl; Tel.: +48-42-2711141; Fax: +48-42-2711140

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Abstract: The growth processes in children depend on the proper functioning of some hormones and growth factors. Recently, a positive correlation between ghrelin and TSH (thyroid stimulating hormone) in patients with hyper- and hypothyroidism was proved. Moreover, in hypothyroid rats with high ghrelin concentration, growth hormone (GH) and insulin-like growth factor I (IGF-I) secretion was suppressed. We analyzed these relationships in euthyroid prepubertal children with idiopathic short stature (ISS). The analysis comprised concentration of ghrelin, GH in stimulating tests and during the night, as well as IGF-I, TSH, free thyroxine (FT4) and free triiodothyronine (FT3) in 85 children with ISS (36 girls, 49 boys) aged 9.65 ± 3.02 years (mean ± SD). A strong positive correlation between ghrelin and TSH was confirmed ($r = +0.44$, $p < 0.05$). A higher ghrelin but lower nocturnal GH and lower IGF-I were observed in children with higher normal TSH concentration than those in children with lower normal TSH. Interestingly, alterations of TSH level were without any impact on FT4 and FT3 concentrations. Summing up, in ISS prepubertal euthyroid children, ghrelin and TSH secretion are closely related. On the other hand, the higher the TSH, the lower the nocturnal GH and IGF-I levels. The contribution of the above findings in deterioration of growth processes requires further studies.

Keywords: ghrelin; growth hormone; thyroid stimulating hormone; free thyroxine; free triiodothyronine; insulin-like growth factor I; idiopathic short stature; children

1. Introduction

Human postnatal growth processes are determined by the proper action of two axes: growth hormone (GH)—insulin-like growth factor I (IGF-I) and thyroid stimulating hormone (TSH)—thyroid hormones (thyroxine—T4 and triiodothyronine—T3). GH regulates cell growth, differentiation, apoptosis, and reorganization of the cytoskeleton; these effects are mediated through IGF-I, which is produced mainly in the liver in response to GH [1]. GH secretion is stimulated directly by GH releasing hormone (GHRH), as well as by ghrelin; the latter hormone additionally can exert an indirect effect [2]. It is worth mentioning that ghrelin is also an orexigenic hormone involved in energy balance regulation [3,4]. In turn, thyroid hormones regulate growth of long bones, protein synthesis, as well as the neuronal proliferation, migration and maturation; they also increase the basal metabolic rate (BMR).

Mutual relationships between growth-related molecules of the above mentioned axes are observed. Free T4 (FT4) and free T3 (FT3) exert a permissive impact on IGF-I action; it was demonstrated that

hypothyroidism, even in its subclinical form, affected IGF-I secretion [5]. Moreover, in short children with FT4 levels within the lowest third of normal range, administration of L-thyroxine (L-T4) improved growth rate and IGF-I response to GH [6]. On the other hand, GH influences the monodeiodination of FT4 to FT3; this reaction is also mediated by IGF-I [7]. In many children with GH deficiency, shortly after the start of GH replacement therapy, FT4 levels significantly decreased [8,9].

Therefore, in the diagnostic process of children with short stature, the presence of GH deficiency, primary IGF-I deficiency, hypothyroidism, as well as the chronic disorders which cause the secondary IGF-I deficiency, should be taken into consideration [10,11]. After these disorders (as well as other known causes of short stature, e.g., genetic disorders) are excluded, an idiopathic short stature (ISS) can be suspected [10] and further watchful waiting is recommended. Unfortunately, some of these children do not achieve predicted final height.

It was reported that in about 40% of children with ISS, a reduced IGF-I concentration was observed, due to unknown causes [12]. Thus, the pathomechanism of the growth processes is still not well explained. In our previous studies, we also noted that among children with ISS, there were a lot of cases with reduced IGF-I concentration [13]. Moreover, the lower the IGF-I was observed, the higher ghrelin concentration was confirmed in this group of children. In ISS group we also found the tendency for ghrelin concentration being higher than in controls [14].

On the other hand, the correlations between ghrelin and TSH or FT4 levels in patients with hypo- and hyperthyroidism have been studied in numerous analyses. It has been documented that ghrelin level is elevated in severe hypothyroidism and reduced in hyperthyroidism, when compared to controls [15]. Thus, in such cases, ghrelin concentration is negatively correlated with FT4 and FT3 and positively with TSH. It has also been shown that ghrelin levels increase when patients with hyperthyroidism develop hypothyroidism after ^{131}I radioiodine therapy [16,17].

Chang et al. observed that in hypothyroid rats with confirmed high ghrelin concentration, GH level was not elevated, while IGF-I secretion was even suppressed [18]. However, it should be stressed that studies concerning the relationships among hormones that are crucial for the growth processes in a group of ISS children have so far been scarce.

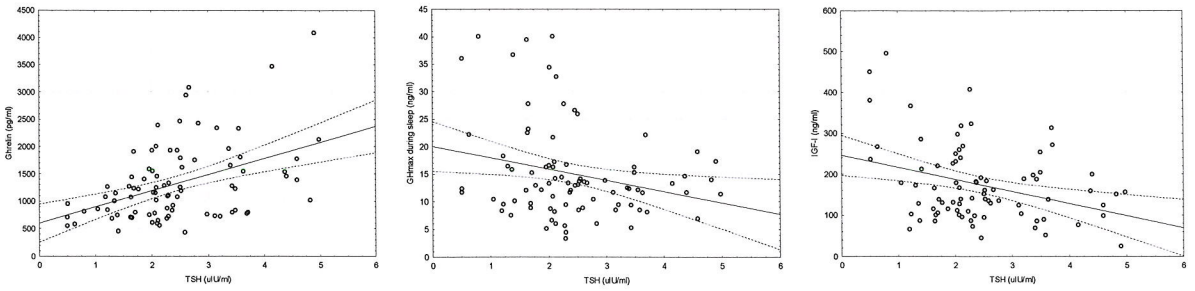
Thus, the aim of the present study has been to analyze the mutual relations among ghrelin, GH, IGF-I, TSH, FT4 and FT3 in ISS euthyroid children (non-GH-deficient); three (3) problems to be elucidated have arisen: (1) whether elevated levels of ghrelin (observed in some ISS children) have any effect on TSH or FT4 and FT3 secretion, the phenomenon which may be responsible for their slow growth velocity; (2) whether the ISS children with FT4 concentration in the lower normal range are characterized by reduced IGF-I and/or by increased ghrelin concentration; and (3) should L-T4 treatment be recommended for them.

2. Results

In the analyzed group of prepubertal euthyroid children with ISS, a significant positive correlation between ghrelin and TSH concentration was confirmed; however, a correlation between ghrelin and FT4 (or FT3) concentration was not noticed. Moreover, a significant negative correlation between TSH and maximal GH concentration during sleep and between TSH and IGF-I concentration was observed (Figure 1).

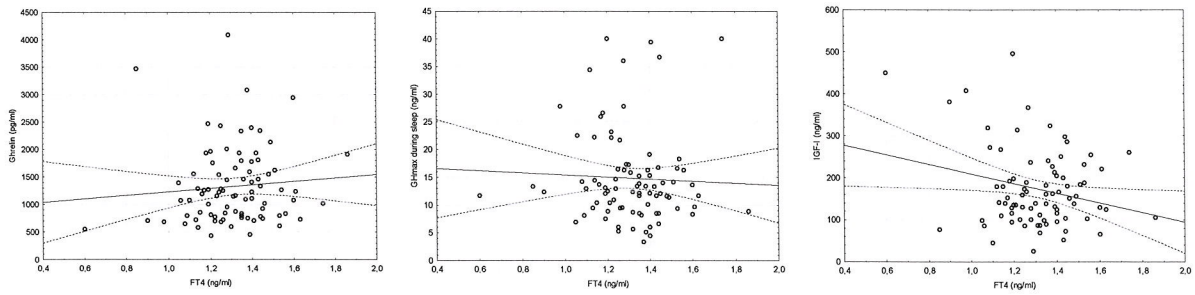
Correlations between TSH and maximal GH levels in individual stimulation tests, or between TSH and FT4 (or FT3) levels, were not observed. On the other hand, we did not find any significant correlations between FT4 levels and ghrelin concentration, maximal GH concentration during sleep or IGF-I concentration (Figure 2).

In turn, the significant negative correlation between ghrelin and IGF-I levels as well as between ghrelin levels and IGF-I/IGFBP-3 (insulin-like growth factor binding protein-3) molar ratio were noticed (Figure 3).



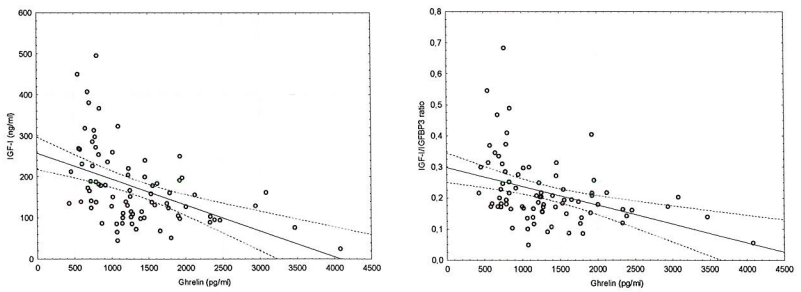
(**a**) (**b**) (**c**)

Figure 1. Correlation between: (**a**) TSH (thyroid stimulating hormone) and ghrelin concentrations (r = +0.44, $p < 0.05$); (**b**) TSH concentration and GHmax concentration during sleep (r = −0.25, $p < 0.05$); (**c**) TSH and IGF-I concentrations (r = −0.3, $p < 0.05$).



(**a**) (**b**) (**c**)

Figure 2. Correlation between: (**a**) FT4 and ghrelin concentrations (r = +0.08, $p > 0.05$); (**b**) FT4 and GHmax during sleep (r = −0.04, $p > 0.05$); (**c**) FT4 and IGF-I concentrations (r = −0.19, $p > 0.05$).



(**a**) (**b**)

Figure 3. Correlation between: (**a**) ghrelin and IGF-I concentrations (r = −0.47, $p < 0.05$); (**b**) ghrelin concentration and IGF-I/IGFBP-3 molar ratio (r = −0.27, $p < 0.05$).

Taking into consideration the positive correlation between ghrelin and TSH levels, we decided to analyze our group of children after dividing them into two subgroups based on TSH median value: children with TSH value below 2.29 μIU/mL (lower normal TSH) and children with TSH value ≥ 2.29 μIU/mL (higher normal TSH). Higher ghrelin concentration but lower IGF-I and GH levels during the night were confirmed in children with TSH concentration equal to or above 2.29 μIU/mL when compared to children with TSH below 2.29 μIU/mL. Interestingly, there were no differences in FT4 and FT3 concentrations between the subgroups (Table 1).

Table 1. The auxologic and biochemical parameters in children with ISS, divided into two (2) groups distinguished by dependence on TSH concentration result: first one higher than or equal to the median value - 2.29 μIU/mL and second lower than 2.29 μIU/mL.

	Lower Normal TSH TSH < 2.29 μIU/mL	**Higher Normal TSH TSH ≥ 2.29 μIU/mL**	*p*
N (f/m)	43 (22/21)	42 (14/28)	
CA (years)	9.43 ± 2.98	8.85 ± 2.87	0.1147
Height SDS	−2.65 ± 0.92	−2.43 ± 0.69	0.2135
BMI SDS	−0.30 ± 1.09	−0.39 ± 0.71	0.6395
TSH (μIU/mL)	1.65 ± 0.51 *	3.22 ± 0.82 *	0.0000
FT4 (ng/mL)	1.30 ± 0.22	1.32 ± 0.16	0.6020
FT3 (pg/dL)	3.89 ± 0.56	4.16 ± 0.37	0.1993
FT4/FT3 ratio	0.34 ± 0.05	0.34 ± 0.04	0.7459
GHmax after clonidine (ng/mL)	19.56 ± 10.33	16.67 ± 7.91	0.1548
GHmax after glucagone (ng/mL)	12.15 ± 7.65	10.31 ± 7.09	0.2757
GHmax during sleep (ng/mL)	17.56 ± 10.31 *	12.46 ± 4.91 *	0.0048
IGF-I (ng/mL)	198.40 ± 107.33 *	148.13 ± 67.42 *	0.0144
IGFBP-3 (μg/dL)	4.59 ± 0.98	4.40 ± 1.58	0.5041
IGF-I/IGFBP-3 ratio	0.24 ± 0.11	0.20 ± 0.10	0.0944
Ghrelin (pg/mL)	1062.36 ± 443.43 *	1578.23 ± 807.37 *	0.0004
Leptin (ng/mL)	5.73 ± 8.09	3.50 ± 5.39	0.1583
Adiponectin (ng/mL)	18.10 ± 8.41	16.72 ± 7.27	0.4416
Resistin (ng/mL)	10.02 ± 3.89	10.27 ± 4.22	0.7856
AR index	0.76 ± 0.27	0.79 ± 0.25	0.5408
Triglycerides (mg/dL)	71.03 ± 26.54	64.57 ± 29.19	0.3729
Cholesterol (mg/dL)	159.73 ± 40.60	162.27 ± 29.72	0.7761
LDL-cholesterol (mg/dL)	92.30 ± 36.32	89.93 ± 25.52	0.7737
HDL-cholesterol (mg/dL)	57.83 ± 15.32	57.39 ± 16.87	0.9162
OGTT, n = 28 (f/m)	12 (6/6)	16 (8/8)	
Glucose 0′ (mg/dL)	82.32 ± 9.19	83.66 ± 9.55	0.5481
Glucose 60′ (mg/dL)	115.25 ± 36.51	137.31 ± 49.29	0.2039
Glucose 120′ (mg/dL)	91.00 ± 20.60 *	120.69 ± 28.73 *	0.0053
Insulin 0′ (μIU/mL)	5.72 ± 3.93 *	3.56 ± 1.90 *	0.0096
Insulin 60′ (μIU/mL)	33.34 ± 20.80	34.15 ± 16.24	0.9081
Insulin 120′ (μIU/mL)	20.15 ± 12.21 *	31.04 ± 15.12 *	0.0481
IRI HOMA	1.20 ± 0.84 *	0.76 ± 0.44 *	0.0158
IRI Belfiore	0.93 ± 0.37	1.05 ± 0.43	0.4570

CA—chronological age; BMI—body mass index; SDS—standard deviation score; IGF-I—insulin-like growth factor I; IGFBP-3—insulin-like growth factor binding protein-3; IRI—insulin resistance index; HOMA—homeostasis model assessment, AR index—adiponectin-resistin index; OGTT—oral glucose tolerance test; *—pairs of results marked with asterisks are characterized by a statistically significant difference; N—number of patients; *p*—level of statistical significance.

A multivariate analysis shows that age, sex and body mass have no impact on this relationship. A higher fasting insulin and IRI HOMA in the group of children with lower normal TSH vs. upper normal TSH was noticed, while glucose and insulin concentration at the 120th minute of OGTT was significantly higher in children with higher vs. lower TSH. No differences were observed between the groups regarding lipids, leptin, resistin or adiponectin.

Next, based on FT4 value, we divided the analyzed group of children into two subgroups: children with FT4 results remaining in the lowest tercile of the reference range and children with FT4 concentration equal to or being above the upper limit of lowest tercile (i.e., remaining in the middle or highest tercile of the reference range). There were no significant differences in the auxologic parameters (growth deficiency or BMI), ghrelin, GH, IGF-I and/or TSH concentration. No differences between groups in regards to glucose, insulin, lipids, leptin, resistin, adiponectin were observed, either. The significant differences in FT4 and FT3 concentration result from the essence of the division used (Table 2).

Table 2. The auxologic and biochemical parameters in children with ISS divided into two (2) groups distinguished by dependence on FT4 concentration: the first one—FT4 remaining in the lowest tercile of the reference range (i.e., below 1.1 ng/mL) and the second—FT4 equal to or being above the upper limit of lowest tercile - 1.1 ng/mL (i.e., FT4 within the middle or highest tercile of the reference range).

	Lower Normal FT4 FT4 < 1.1 ng/mL	**Higher Normal FT4 FT4 ≥ 1.1 ng/mL**	*p*
N	21	64	
CA (years)	9.80 ± 3.27	9.54 ± 2.94	0.7382
Height SDS	−2.74 ± 0.52	−2.49 ± 0.88	0.2364
BMI SDS	−0.44 ± 0.93	−0.31 ± 0.92	0.5978
TSH (μIU/mL)	2.16 ± 1.11	2.52 ± 1.02	0.1792
FT4 (ng/mL)	1.07 ± 0.15	1.3 ± 0.13	0.0000
FT3 (pg/dL)	3.50 ± 0.71	4.13 ± 0.36	0.0147
FT4/FT3 ratio	0.34 ± 0.06	0.34 ± 0.04	0.9778
GHmax after clonidine (ng/mL)	15.76 ± 7.63	18.84 ± 9.75	0.2007
GHmax after glucagone (ng/mL)	11.04 ± 6.54	11.34 ± 7.79	0.8744
GHmax during sleep (ng/mL)	15.97 ± 7.76	14.47 ± 8.46	0.4825
IGF-I (ng/mL)	191.05 ± 118.11	167.01 ± 83.72	0.3214
IGFBP-3 (μg/dL)	4.49 ± 0.90	4.50 ± 1.42	0.9817
IGF-I/IGFBP-3 ratio	0.23 ± 0.13	0.21 ± 0.10	0.4081
Ghrelin (pg/mL)	1224.93 ± 712.56	1357.95 ± 691.69	0.4581
Leptin (ng/mL)	5.12 ± 8.60	4.54 ± 6.55	0.7633
Adiponectin (ng/mL)	14.98±6.38	18.07 ± 8.19	0.1548
Resistin (ng/mL)	9.49 ± 4.13	10.27 ± 4.03	0.4841
AR index	0.80 ± 0.20	0.77 ± 0.28	0.6998
Triglycerides (mg/dL)	77.13 ± 36.27	64.41 ± 23.69	0.1186
Cholesterol (mg/dL)	172.33 ± 47.79	156.65 ± 29.93	0.1137
LDL-cholesterol (mg/dL)	103.13 ± 44.56	86.67 ± 23.73	0.0718
HDL-cholesterol (mg/dL)	56.25 ± 13.22	58.12 ± 16.98	0.6922
OGTT, n = 28 (f/m)	9 (5/7)	19 (9/7)	
Glucose 0′ (mg/dL)	84.56 ± 6.96	82.28 ± 10.01	0.3763
Glucose 60′ (mg/dL)	139.57 ± 48.49	123.95 ± 44.19	0.4358
Glucose 120′ (mg/dL)	110.29 ± 35.53	107.19 ± 27.81	0.8136
Insulin 0′ (μIU/mL)	4.30 ± 3.61	4.51 ± 2.90	0.8200
Insulin 60′ (μIU/mL)	34.85 ± 24.59	33.41 ± 16.20	0.8597
Insulin 120′ (μIU/mL)	26.15 ± 19.81	25.93±13.15	0.9741
IRI HOMA	0.93 ± 0.86	0.94 ± 0.58	0.9614
IRI Belfiore	0.83 ± 0.55	1.05 ± 0.33	0.2136

CA—chronological age; BMI—body mass index; SDS—standard deviation score; IGF-I—insulin like growth factor I; IGFBP-3—insulin-like growth factor binding protein 3; IRI—insulin resistance index; HOMA—homeostasis model assessment, AR index—adiponectin-resistin index; OGTT—oral glucose tolerance test; N—number of patients; *p*—level of statistical significance.

Similarly, when we divided our group into 2 subgroups according to FT4 median value (1.3 ng/mL; the first group below the median value, the second equal to or above 1.3 ng/mL), no differences for any of the analyzed parameters were found.

3. Discussion

In our present study, we have attempted to clarify the relationships between two growth regulatory feedback loops: ghrelin-GH-IGF-I and TSH-FT4/FT3 in euthyroid short children diagnosed with ISS. The initial point for us was the observation that in about 40% of children in whom "idiopathic" short stature was diagnosed, low IGF-I concentrations were observed [12]. In our earlier study, we also confirmed these findings [13]. In our research, we considered various causes of low IGF-I (secondary IGF-I deficiency), one of them being the reduction in IGF-I production as a result of chronic disorders, mainly gastrointestinal tract diseases or malnutrition (due to the mechanism of sirtuins dysfunction). For this reason, the exclusion criteria included malnutrition and a history of chronic diseases, as well as abnormalities found during physical examination. Therefore, we believe that the cause may be a disturbance of the GH secretagogue type 1a receptor or GH receptor activity or others. Moreover, we also showed a negative correlation between IGF-I and ghrelin concentration [19]. As ghrelin is a

stimulating factor for GH secretion, and, in turn, GH stimulates IGF-I secretion in healthy children, we have concluded that in short children a low concentration of IGF-I triggers feedback mechanisms, i.e., higher production of ghrelin. The causes of low IGF-I secretion observed in these children are not fully understood and other mechanisms responsible for this phenomenon should be sought.

In most human studies based on the results obtained from patients with hyperthyroidism and hypothyroidism, a positive correlation between ghrelin and TSH was confirmed [15–17,20]. In our present study, we have also confirmed a strong positive correlation between TSH and ghrelin concentration in the group of children that we analyzed. However, it should be emphasized that our study included only euthyroid children. The authors cited above have suggested that the fluctuation of ghrelin secretion acts as a compensatory factor, helping to balance metabolic disturbances in patients with hyperthyroidism and hypothyroidism. However, it is to be recalled that short children analyzed by us, were euthyroid. Therefore, the explanation for these findings seems to be different.

It has been proved that ghrelin is able to stimulate TSH from thyrotropic cells of anterior pituitary and the density of ghrelin receptors seems to increase in mice when the food intake is not sufficient [21]. In our previous study, we noticed that ghrelin secretion was higher in slim children, compared to children with normal body weight [19]. It is well known that ghrelin, apart from being a GH stimulatory factor, is also an orexigenic hormone that regulates appetite and affects metabolic homeostasis [3,4]. Thus, in contrast to GH, ghrelin secretion is expressed not only during the night but also during the day and its production depends on food intake: it increases in the fasting state and decreases after meals [22,23]. Moreover, ghrelin levels negatively correlate with body mass: its concentration increases in malnutrition and decreases in obesity [24,25]. Although a negative correlation between ghrelin concentration and BMI SDS was found in the analyzed group of ISS children, when we divided them into two subgroups (one with low normal TSH and one with high normal TSH) we did not observe any differences as regards the nutritional status of the children, expressed by BMI SDS. Therefore, the nutritional status of the children was not a significant factor in the issue under consideration. It was also confirmed by the results of the multivariate analysis.

Since we observed a positive correlation between ghrelin and TSH concentration, we assumed that increased ghrelin level (which is probably a response to low IGF-I formation) stimulated the release of TSH from the pituitary gland. Thus, it was surprising that we did not observe any effect on FT4 levels. Ghrelin receptors were also found on human thyrocytes; the indirect suppression effect of ghrelin on thyrocytes was documented by Barington et al. in 2017 [26]. The authors have concluded that ghrelin possesses the ability to reduce TSH-induced thyroglobulin level through the deterioration of thyroid peroxidase (TPO) expression. Therefore, we speculate that this may be a reason why FT4 concentration is not elevated, despite higher TSH concentration. Furthermore, no correlation between ghrelin and FT4 secretion, or between FT4 and IGF-I secretion, was found. It is an important issue for further consideration. Hypothyroidism, even in its subclinical form, is known to affect IGF-I [5] secretion and the administration of L-thyroxine (L-T4) improves growth rate and IGF-I response to GH in short children with lower normal FT4 levels [6]. Thus, based on the results of our analysis, we did not find objective evidence that the application of L-T4 treatment in children with low normal FT4 effectively improved their growth rate, just as we did not observe any correlation between FT4 and IGF-I.

In our study we also analyzed the FT4/FT3 ratio. Although the effect of IGF-I on deiodination of FT4 to FT3 is well known, in the group of children we have analyzed, both FT4 and FT3 levels have not differed between the group with higher ghrelin and lower IGF-I and the group with lower ghrelin and higher IGF-I. About 80% of the deiodination takes place in peripheral organs, such as the liver and kidney, and occurs intracellularly, while the concentrations measured in the blood represent the level of FT4 and FT3 secretion from the thyroid gland, which is about 20% of deiodination. We believe that this is the reason why the FT4/FT3 ratio does not differ between groups.

It is interesting that the higher the TSH and ghrelin are, the lower the GH concentration is during the night and the lower the IGF-I. Boulenger et al. [27] have proved that in hypothyroid rats, GHRH receptors in the anterior pituitary gland are down-regulated, which may result in a

reduced GH production after GHRH stimulation. A similar relationship was also observed by Chang et al. [18]. They analyzed the results of TSH, ghrelin, GH and IGF-I in rats, divided into four groups, according to four (4) procedures performed: thyroidectomy (Tx), sham Tx, Tx + L-T4 therapy and after propylothiouracyl (PTU) injection. The authors observed that in rats, both after Tx and PTU (i.e., with primary hypothyroidism and with higher TSH), ghrelin increased by 75% and GH secretagogues receptor type 1 (GHS-R1) expression in the anterior pituitary was up-regulated. However, in both groups with hypothyroidism, similarly to the results of our study, it did not cause a significant increase in GH secretion, while IGF-I concentration decreased by 51% and 63%, respectively.

In our study, as in the report by Chang et al. [18], we also observed differences between IGF-I concentration in individual groups; IGF-I was negatively correlated with TSH and ghrelin, positively with nocturnal GH. Surprisingly, this correlation was not observed for the GH results obtained during GH stimulation tests, routinely used in GHD diagnostics.

Chang et al. [18] concluded that hypothyroidism disrupts ghrelin/GHS-R axis for stimulating GH secretion. The relationships presented by authors in the mentioned study [18] concerned cases with hypothyroidism, while the group of children we analyzed did not have a thyroid gland dysfunction. However, some analogies arise. It seems that it is worth discussing the normal range for TSH concentration in children with ISS, since after L-T4 administration in young adult rats, in Chang et al.'s study [18], ghrelin concentration decreased, GH secretion was enhanced and IGF-I concentration normalized.

Summing up, we observed a positive correlation between ghrelin and TSH in euthyroid children with idiopathic (non-GH deficit) short children. It was confirmed that the higher the ghrelin and TSH, the lower the GH secretion during the night and the lower the IGF-I. However this is without any impact on FT4 concentrations.

We speculate that in children with ISS in whom ghrelin concentration is elevated (probably in response to low IGF-I secretion), TSH impact on FT4 secretion from thyrocytes is reduced by higher ghrelin concentration. In the feedback mechanism, increasing secretion of TSH from the pituitary gland for the purpose of FT4 normalization is observed.

The second hypothesis is that due to higher ghrelin concentration, the stimulation of TSH secretion from pituitary gland is enhanced. However, due to a weaker impact of TSH on thyrocytes under this condition (high ghrelin concentration), the FT4 concentration is not elevated and stays at the same level as in children with lower TSH.

It is possible that relative hypothyroidism disrupts the ghrelin/GHS-R axis impact on stimulating GH secretion, as GH secretion at night is disturbed in these children (while GH results obtained during GH stimulation tests, routinely used in GHD diagnostics, are normal). Further studies are needed to explain these findings.

Limitation of the study: Certainly, employing a control group consisting of healthy children with normal height would have facilitated the interpretation of the results. In addition, generally accepted reference values for ghrelin concentration are not yet available. Stimulation tests for GH secretion are still the basic tool in the diagnosis of GHD, however when drawing conclusions from the test results, it must be taken into account that their reproducibility creates some problems.

4. Materials and Methods

The study was approved by the Bioethical Committee at the Polish Mother's Memorial Hospital - Research Institute (PMMH-RI) in Lodz.

The children were recruited over a period of 18 months from patients of the Outpatient Clinic of PMMH-RI, where they had been referred due to short stature. In all the children, height and body mass were measured, using a stadiometer and scales, respectively. Next, the height standard deviation score (height SDS) and body mass index standard deviation score (BMI SDS) were calculated, based on the current population standards data, given by Palczewska and Niedźwiecka [28]. The stage of puberty was assessed according to the Tanner's scale [29]. Next, a detailed medical history was collected

and the thyroid function was assessed in each child, based on TSH and FT4 serum concentrations. All children qualified for the study lived in areas of sufficient iodine supply.

Exclusion criteria: (1) –height SDS above −2.0 SD, (2) undernutrition and obesity (BMI SDS above +2.0 SD or below −2.0 SD for the reference range for age and sex [28]), (3) puberty stage more than Tanner I stage, (4) history of chronic diseases, dysmorphic features or abnormalities found during the physical examination, (5) abnormal results of TSH and/or FT4.

Next, the children were diagnosed at the Department of Endocrinology and Metabolic Diseases of PMMH-RI, to assess GH secretion and IGF-I concentrations.

In each child, a 3-h, nocturnal profile of GH secretion was recorded every half-hour, starting from the first hour after falling asleep.

Then, two GH-stimulation tests were performed on subsequent days of hospitalization. In the first of them, GH was measured before (0 point) and at the 30th, 60th, 90th and 120th minute after oral clonidine administration in dose of 0.15 mg/m^2 of body surface. In the second one, GH was measured before (0 point) and at the 90th, 120th, 150th and 180th minute after intramuscular glucagon administration in dose of 30 μg/kg (not exceeding 1 mg). Peak GH concentration (GH_{max}) was determined in both tests and after falling asleep. The cut-off value for normal and subnormal GH peak in response to stimulation was 10.0 ng/mL, according to current recommendations [30,31].

In children with GH_{max} values < 10 ng/mL, GHD was recognized and those cases were excluded from further analysis, while in children with normal value of GH_{max} (more than 10 ng/mL), ISS was diagnosed and those cases were qualified into the study group.

Finally, 85 children with ISS (36 girls and 49 boys), aged 9.65 ± 3.02 years (mean ± SD), from 4.6 to 12.4 years were selected for the study group.

In each child, in the morning, in fasting state, ghrelin, IGF-I, IGFBP-3, TSH, FT4, FT3, anti-tyreoperoxidase (aTPO) and anti-tyreoglobulin (aTg) antibodies, lipids, glucose, insulin, leptin, adiponectin and resistin concentrations were measured in single blood samples. Additionally, in 28 children, oral glucose tolerance tests (OGTT) were performed including an assessment of glucose and insulin at 0′, 60′ and 120′ minutes from oral glucose administration (dose 1.75 g/kg body mass, max. 75 g).

The concentrations of GH were measured by a two-site chemiluminescent enzyme immunometric assay (hGH IMMULITE, DPC) for the quantitative measurement of human GH, calibrated to WHO IRP 80/505 standard. The analytical sensitivity of the assay was up to 0.01 ng/mL, the calibration range up to 40 ng/mL, the sensitivity of 0.01 ng/mL, the intra-assay coefficient of variation (CV) of 5.3–6.5% and the inter-assay CV of 5.5–6.2%.

The total ghrelin concentration was measured using the Millipore RIA kit (Linco Research, St. Charles, MO, USA) with sensitivity level: 100–10.000 pg/mL, the intra-assay CV: 3.3–10.0% and inter-assay CV: 14.7–17.8%.

Both IGF-I and IGFBP-3 concentrations were assessed with Immulite, DPC assays. For IGF-I, WHO NIBSC 1st IRR 87/518 standard was applied, with the analytical sensitivity of 20 ng/mL, calibration range up to 1600 ng/mL, intra-assay CV: 3.1–4.3% and inter-assay CV: 5.8–8.4%. The assay for IGFBP-3 assessment was calibrated to WHO NIBSC Reagent 93/560 standard, with analytical sensitivity 0.02 μg/mL, the calibration range up to 426 μg/mL, the intra-assay CV: 3.5–5.6% and the total CV: 7.5–9.9%.

For the calculation of IGF-I/IGFBP-3 molar ratio, the following molecular masses were used: 7.5 kDa for IGF-I and 42.0 kDa for IGFBP-3 [32,33].

Serum TSH, FT4 and FT3 concentrations were measured by the electroimmuno-chemiluminescent method (ECLIA), Roche, Elecsys ® Systems 1010/2010/modular analytics E170. For TSH, the analytical sensitivity was 0.005 μIU/mL, range up to 100 μIU/mL, intra-assay coefficient of variance (CV) 1.5–8.6%, accuracy 1.1–3.0%. The analytical range for FT4 was 0.023–7.77 ng/mL, intra-assay CV 1.4–2.9%, accuracy 2.7–6.6%. For FT3, the analytical range was 0.26–32.55 pg intra-assay CV 3.7–9.5%, accuracy

3.8–11.2%. For the assessment of FT3/FT4 molar ratio, the concentrations of FT4 and FT3 were expressed as molar ones.

The leptin, resistin and adiponectin concentrations were measured using the Millipore ELISA kit (Linco Research). The sensitivity level, the intra-assay CV and inter-assay CV were: 0.5–100 ng/mL, 1.4–4.9% and 1.3–8.6% for leptin; from 0.16 ng 3.2–7.0% and 7.1–7.7% for resistin and from 0.78 ng/mL, 7.4% and 2.4–8.4% for adiponectin, respectively. Based on fasting adiponectin and resistin concentrations, the adiponectin-resistin (AR) index, according to the formula proposed by Lau and Muniandy [34], was calculated: 1+log10 (fasting resistin)-log10 (fasting adiponectin).

Plasma insulin concentration was measured using the DRG ELISA kit; sensitivity level 1.76 – 100 μIU/mL, the intra-assay CV: 1.8–2.6 and inter-assay CV: 2.9–6.0. Plasma glucose concentration was determined using the enzymatic method, with the use of hexokinase.

Based on the results of fasting glucose and insulin concentration, insulin resistance index was calculated [35], according to the homeostasis model assessment (IRI HOMA): fasting glucose [mmol/L] x fasting insulin [μIU/mL])/22.5.

Based on the glucose and insulin concentration during OGTT, $IRI_{Belfiore}$ was calculated according to the formula [36] = 2/{[1/(GLU_{AUC} x INS_{AUC})]+1}, where: GLU_{AUC} = $GLU_{AUCi}/GLU_{AUCmean}$, while INS_{AUC} = $INS_{AUCi}/INS_{AUCmean}$, where GLU_{AUCi} and INS_{AUCi}—areas under respective glucose or insulin concentration curve during OGTT in a given patient, while $GLU_{AUCmean}$ and $INS_{AUCmean}$—areas under respective glucose or insulin concentrations during OGTT for an age group for our population (those values were calculated in our earlier study) [37].

The data were analyzed using Statistica 11.0 software (StatSoft, Inc., Tulsa, OK, USA). The continuous variables were expressed as mean ± standard deviation for normally distributed variables. Shapiro–Wilk's test was used to test the distribution of the variables. The differences between the sexes were compared using chi^2 test. Correlations were evaluated using the Pearson's test. A one-way ANOVA was applied for statistical analysis with the subsequent use of a post-hoc test, in order to statistically assess differences between groups; Tukey's test was selected because of the uneven amount of data in individual groups. $p < 0.05$ was accepted as significant value.

5. Conclusions

In children with ISS, TSH and ghrelin secretion are closely related and a positive correlation between their concentrations was observed. It is interesting that the higher ghrelin and TSH concentrations, the lower the nocturnal GH and IGF-I secretion, however, without any impact on FT4 concentration.

It seems that ISS children in whom the FT4 concentration value is in the lowest tercile of the normal range, as well as ISS children with TSH level in the upper normal range (above the median value), do not require treatment with L-T4.

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6.3 Leptin does not stimulate TSH secretion in obese short children

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Leptin Does Not Influence TSH Levels in Obese Short Children

Katarzyna Adamczewska[1], *Zbigniew Adamczewski*[2], *Andrzej Lewiński*[3,4] *and Renata Stawerska*[3,5*]

[1] Primary Care Unit "Medyk" – Outpatient Clinic, Ozorków, Poland, [2] Department of Nuclear Medicine, Medical University of Lodz, Lodz, Poland, [3] Department of Endocrinology and Metabolic Diseases, Polish Mother's Memorial Hospital-Research Institute, Lodz, Poland, [4] Department of Endocrinology and Metabolic Diseases, Medical University of Lodz, Lodz, Poland, [5] Department of Pediatric Endocrinology, Medical University of Lodz, Lodz, Poland

Introduction: Growth hormone (GH) and thyroid hormones are important for children growing. In some obese children a slightly elevated TSH concentration is observed. This may be an adaptive mechanism: stimulation of pro-TRH biosynthesis in the hypothalamus in response to elevated leptin. The increased TSH may also reflect the necessity of maintaining the resting energy expenditure or may be a result of inappropriate, low FT4 concentration. Thus, we evaluated serum TSH and FT4 concentrations in idiopathic short stature (ISS) children (non GH-deficient) and examined the effect of children's nutritional status and levels of selected adipocytokines on thyroid function, searching for the presence of various forms of subclinical hypothyroidism, which may be the cause of the slow growth rate.

Methods: The study group included 115 children (50 girls and 65 boys) with ISS, aged (mean ± SD) 10.4 ± 3.34 years. In each child, lipids, TSH, FT4, IGF-1, maxGH during the stimulation tests, leptin, adiponectin and resistin concentrations were determined. Based on BMI SDS, 3 subgroups: slim (n=26), obese (n=21) and normal weight (n=68) were distinguished.

Results: There was no correlation between leptin level and TSH, FT4 levels. The levels of leptin, total cholesterol and LDL-cholesterol in obese short children were significantly higher than in children from other subgroups. In turn, the levels of adiponectin, resistin, TSH and FT4 did not differ between subgroups. In 7% of children, an elevated TSH level was found (but less than 10 mIU/L), with a similar frequency across subgroups. The higher the leptin, the lower maxGH in clonidine stimulation test was recorded.

Conclusions: It seems that in obese children with idiopathic short stature leptin does not increase TSH secretion. This may be related to a disruption of the effect of leptin on TSH production and could indicate wide ranging disturbances of hypothalamic signals, and consequently be the cause of inappropriate GH secretion.

Keywords: obesity, children, thyroid stimulating hormone, leptin, idiopathic short stature

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Piotr Miśkiewicz,
Medical University of Warsaw, Poland
Tommaso Aversa,
University of Messina, Italy
Alina German,
Technion Israel Institute of Technology, Israel

***Correspondence:**
Renata Stawerska
renata.stawerska@umed.lodz.pl

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INTRODUCTION

Growth hormone (GH), insulin-like growth factor 1 (IGF-1) and thyroid hormones play a key role in the growth processes in children. They act directly, however free T4 (FT4) and free T3 (FT3) also exert a permissive impact on IGF-1 action (1).

A child's short stature may be due to hormonal causes (growth hormone deficiency - GHD, hypothyroidism or hypercortisolemia), chronic diseases (e.g. gastrointestinal diseases such as celiac disease) or some genetic syndromes (e.g. Turner or Prader-Willi syndrome). However, in many children, despite numerous tests, it is impossible to establish the cause of an inadequate high velocity and short stature. In such cases, idiopathic short stature (ISS) is diagnosed; however, it can be assumed that many of these cases are undiagnosed abnormalities caused by other factors involved in the regulation of the growth process (2).

It is known that many environmental factors and disruptors as well neuropeptides affect the production and secretion of TSH (3, 4). In our previous study, we proved a positive correlation between ghrelin and TSH concentrations in children with ISS. We also demonstrated that the higher the TSH, the lower the nocturnal GH and IGF-1 levels were recorded (5). Now, we have decided to analyse the effect of some selected hormones secreted from the adipose tissue (adipocytokines) on the secretion of TSH and FT4 in ISS children.

Subclinical hypothyroidism is observed in about 2% children (6, 7) and its diagnosis and treatment are a matter of controversy (8, 9); there are some conditions in which TSH elevation is transient, with obesity being one of the well documented examples (10). Many studies report that in children with excess body weight, a slightly elevated TSH level is observed (4-10 mIU/l), although it is not a response to reduced FT4 levels, i.e. not a result of hypothyroidism (10, 11). It has been hypothesized that it may be an adaptive mechanism aimed at increasing energy expenditure and the levels of TSH and FT4 correlate positively with resting energy expenditure (12). Thus, the elevated TSH levels in these cases seem a consequence rather than a cause of obesity, and treatment with levothyroxine in obese children is not recommended (12, 13). However, the causes of increased TSH in obesity are not clear and do not apply to all obese children (14). On the other hand, it was shown that among obese teenagers, the higher the TSH concentration, the higher total cholesterol and blood pressure values, which all are symptoms of hypothyroidism (15).

Therefore, the question arises: is the lack of an increase in TSH concentration in some obese children (without thyroid disease) also a normal phenomenon or, on the contrary, can it be the result of a too weak reaction of the hypothalamus and pituitary gland in TRH production/release in response to the body's needs? In the latter case, abnormal hypothalamic and pituitary responses could explain short stature as an effect of relative FT4 deficiency.

The most popular hypothesis explaining the increase in TSH concentration in obese people is that leptin influences the production of pro-TRH in the hypothalamus (16, 17). Serum leptin concentration is strongly positively correlated with body weight and triggers a lot of actions connected with energy expenditure: at the level of the hypothalamus, it also inhibits appetite and increases hepatic gluconeogenesis and muscle fatty acids oxidation in peripheral tissues (18–20). The results of studies concerning leptin concentrations in children with GHD are divergent (21–24). It is possible that in some of them, the relative leptin resistance is observed. On the other hand, it was shown that leptin levels did not differ between children with hypothyroidism and hyperthyroidism, while significant differences were observed for adiponectin and resistin (respectively, higher and lower concentrations in untreated Graves' disease than in hypothyroidism) (25).

Thus, it seems that the abovementioned adipocytokines (leptin, adiponectin and resistin) produced by adipose tissue may be involved in the cross-talk between adipocytes and hypothalamus, with the aim of increasing the release of TRH and TSH, and - in consequence – the production and secretion of FT4.

The aim of the study was to evaluate TSH and FT4 in idiopathic short stature (ISS) children, and to examine the effect of children's nutritional status and levels of selected adipocytokines on thyroid function, in the search for various forms of subclinical hypothyroidism, which may be the cause of the slow growth rate.

Thus, the primary endpoint was the answer to the question how many children with short stature and obesity had elevated TSH levels co-occuring with elevated leptin levels. And - in those who do not have elevated TSH - is it associated with symptoms of hypothyroidism other than short stature, e.g. elevated cholesterol concentration?

MATERIALS AND METHODS

The study included consecutive children admitted to the Department of Endocrinology and Metabolic Diseases of Polish Mother's Memorial Hospital - Research Institute in Lodz over the period of one year for the diagnostics of their short stature, who met the following criteria:

1. height standard deviation score (HSDS) below -2.0 from the mean value for child's age and sex (children's height was measured using a stadiometer) (26);

2. excluding genetic reasons of the short stature (i.e. Turner syndrome, Prader-Willi syndrome) (assessed on the basis of a karyotype);

3. excluding treated hypothyroidism, chronic diseases or undiagnosed gastrointestinal tract complaints (assessed on the basis of a negative history of chronic diseases, as well as normal tests results of tissue transglutaminase antibodies class IgA).

Out of the initially analyzed group of 170 children: 10 did not meet the criterion of height below 3 centile, 7 did not meet the criterion of low height velocity, 4 had treated hypothyroidism, and 1 - celiac disease. Ultimately, 148 short children were qualified for further analyses.

The body mass was assessed in all patients, and that was followed by a calculation of the body mass index standard deviation score for chronological age (BMI SDS for CA).

We used Polish references (26). Next, in all the children, considering the child's current position on centile charts, the height age (HA) was calculated (as the age ascribed to the 50th percentile for a given child's height) and BMI values reffered to HA and expressed as BMI SDS for HA (we adjusted the results to the height age of a child to avoid false results for short children). Based on this value, the analyzed group of short children was divided into three subgroups (according to WHO recomendatios): obese, normal and slim. The obesity and overweight group includes children aged 5–19 with BMI for HA above +1.0 SD (above 90 percentile) and children under 5 years of age with BMI for HA above +2.0 SD (above 97 percentile). Into the slim group, we qualified children with thinness: BMI SDS for HA <-2.0 SD (below the 3rd percentile), regardless of their chronological age.

The stage of puberty was assessed in each child, using the Tanner's scale. Most of the analyzed children were prepubertal (83%).

In all of them, GH secretion was assessed during a 3-hour nocturnal profile and during two (2) stimulation tests: the first one after clonidine administered orally (with the dose of 0.15 mg/m^2 of the body surface) and GH concentration measurements at time 0 and at the 30th, 60th, 90th and 120th minute of the test, and the second one – after intramuscular administration of glucagon (in the dose of 30 μg/kg of body weight, not exceeding 1 mg), with GH concentration measurements at time 0 and at the 90th, 120th, 150th and 180th minute. Based on the results of GHmax values in these tests, we diagnosed:

1. ISS – correct results in - at least - one of the stimulation tests (GHmax values ≥ 10 ng/ml) in 115 children (50 girls and 65 boys),

2. GHD – decreased GH secretion (GHmax values < 10 ng/ml) in 33 children.

In each child, the morning serum cortisol and ACTH levels were routinely assessed to rule out secondary adrenal insufficiency, while in obese children, also the cortisol profile (or a dexamethasone test) was performed to rule out hypercortisolemia. These disorders were not found in any of the children. We also routinely assessed the levels of anti thyroglobulin (a-Tg) and anti thyroid peroxidase (a-TPO) antibodies, they were normal in every child. In each child, the concentration of IGF-1, IGFBP-3, lipids, TSH, FT4, leptin, adiponectin and resistin was assessed in the fasting state on the first day of hospitalisation, just before the first stimulating test. Next, IGF-1 concentrations were calculated as IGF-1 SDS, according to the reference data (27). For the calculation of IGF-1/IGFBP-3 molar ratio, the following molecular masses were used: 7.5 kDa for IGF-1 and 42.0 kDa for IGFBP-3. For IGF-1/IGFBP-3 molar ratio, the cutoff point was established at the median values.

Growth hormone levels were measured using the immunometric method. The measurements were performed by Immulite, DPC assay kits, calibrated to the WHO IRP 98/574 standard set, of the following sensitivity level: 0.01 ng/ml, range: up to 40 ng/ml, the conversion index: ng/ml x 2.6 = mIU/l, the intra-assay CV: 5.3-6.5% and inter-assay CV: 5.5-6.2%.

Both IGF-1 and IGFBP-3 concentrations were assessed by Immulite, DPC assays; WHO NIBSC 1st IRR 87/518 standard was applied, with the analytical sensitivity of 20 ng/ml, calibration range up to 1600 ng/ml, the intra-assay CV: 3.1-4.3% and inter-assay CV: 5.8-8.4%. The assay for IGFBP-3 assessment was calibrated to WHO NIBSC Reagent 93/560 standard, with analytical sensitivity 0.02 μg/ml, the calibration range up to 426 μg/ml, the intra-assay CV: 3.5-5.6% and the total CV: 7.5 9.9%.

The leptin, resistin and adiponectin concentrations were measured using the Millipore Elisa kit (Linco Research, USA). The sensitivity level, intra-assay CV and inter-assay CV were: 0.5-100 ng/ml, 1.4-4.9% and 1.3-8.6% for leptin; from 0.16 ng/ml, 3.2-7.0% and 7.1-7.7% for resistin and from 0.78 ng/ml, 7.4% and 2.4-8.4% for adiponectin, respectively.

Concentrations of TSH and FT4 were measured by the electrochemiluminescent immunoassays (ECLIA) method with commercially available appropriate kits (Roche Diagnostic, Mannheim, Germany). Normal range values were as follows: for TSH: age-dependent ranges - 1–7 years-0.7–5.97 mIU/l; 7–12 years-0.6–4.84 mIU/l; 12–18 years-0.51–4.4 mIU/l with inter-assay coefficients of variation (CVs) 1.3–1.8% and for FT4: age-dependent ranges-1–6 years-0.96–1.77 ng/dl; 6–11 years-0.97–1.67 ng/dl; 11–18 years-0.98–1.63 ng/dl with CVs 2.0–2.4%.

The data were analyzed using Statistica 11.0 software (StatSoft, Inc., Tulsa, OK, USA). The continuous variables were expressed as mean ± standard deviation for normally distributed variables. Shapiro-Wilk's test was used to test the distribution of the variables. Correlations were evaluated using the Pearson's test. A one-way ANOVA was applied for statistical analysis with the subsequent use of a *post-hoc* test, in order to statistically assess differences between groups; Tukey's test was selected because of the uneven amount of data in individual groups. $p < 0.05$ was accepted as significant value.

RESULTS

Among 115 children (50 girls and 65 boys), aged 3.66 to 16.52 yrs; the mean age ± SD: 10.43 ± 3.34 years with ISS, we found: 26 slim children, 68 – with normal body weight and 21 - overweight or obese. The results of the auxological parameters and the laboratory tests results for individual subgroups (divided by BMI values) are presented in **Table 1**.

TSH levels were slightly elevated in 8 children: including 2 out of 26 slim children (7.7%), 2 out of 21 obese children (9.5%) and 4 out of 68 normal weight children (5.9%); FT4 level was in normal range in each of these cases.

As expected, the levels of leptin in obese children were significantly higher than in the other groups, but the levels of adiponectin and resistin did not differ between groups.

The degree of growth deficiency and the other (except leptin) test results did not differ between subgroups.

Mean FT4 concentration was the lowest in the subgroup of obese children, but the values did not reach statistical significance (**Table 1**).

In the whole group of ISS children, we observed a positive significant correlation between adiponectin and FT4

TABLE 1 | The results of analysed parameters in individual subgroups (slim, normal and obese) of ISS children.

	Slim	Normal	Obese	Variance analysis
n = (girls/boys)	26 (14/12)	68 (25/43)	21 (11/10)	
Chronological age (CA) (years)	9.60 ± 3.12	10.56 ± 3.12	10.26 ± 3.31	ns
Height age (HA) (years)	7.18 ± 2.38	8.29 ± 2.62	7.59 ± 2.70	ns
HSDS	-2.71 ± 0.97	-2.53 ± 0.82	-2.53 ± 0.88	ns
BMI (kg/m^2)	13.41 ± 0.80^{a,b}	16.33 ± 1.44^{a,c}	20.40 ± 2.94^{b,c}	<0.0005
BMI SDS for CA	-1.28 ± 0.42^{a,b}	-0.46 ± 0.48^{a,c}	1.43 ± 0.95^{b,c}	<0.0005
BMI SDS for HA	-1.27 ± 0.34^{a,b}	-0.03 ± 0.41^{a,c}	2.05 ± 1.01^{b,c}	<0.0005
TSH (mIU/l)	2.71 ± 1.30	2.53 ± 1.14	2.69 ± 1.52	ns
FT4 (ng/ml)	1.36 ± 0.12	1.31 ± 0.20	1.3 ± 0.14	ns
TG (mg/dl)	74.05 ± 27.32	64.85 ± 25.07	74.29 ± 31.54	ns
CH (mg/dl)	150.00 ± 34.72	160.00 ± 28.39	175.56 ± 49.46	<0.05
LDL-CH (mg/dl)	83.58 ± 28.25^{a}	85.62 ± 26.62^{b}	108.21 ± 36.71^{a,b}	<0.05
HDL-CH (mg/dl)	55.58 ± 19.26	60.72 ± 16.54	60.79 ± 11.91	ns
HDL/CH	0.36 ± 0.10	0.39 ± 0.09	0.34 ± 0.08	ns
Leptin (ng/ml)	2.44 ± 5.11^{a}	4.59 ± 5.04^{b}	11.86 ± 11.47^{a,b}	<0.0005
Adiponectin (ng/ml)	18.43 ± 6.97	17.77 ± 8.30	19.80 ± 11.43	ns
Resistin (ng/ml)	9.91 ± 4.05	10.48 ± 4.01	9.13 ± 2.31	ns
Leptin/Adiponectin ratio	0.07 ± 0.4^{a}	0.35 ± 0.4^{b}	0.73 ± 0.43^{a,b}	<0.0005
maxGH after clonidine (ng/ml)	19.50 ± 9.59^{a}	17.43 ± 8.70^{b}	11.75 ± 6.63^{a,b}	<0.05
maxGH after glucagon (ng/ml)	10.81 ± 7.80	9.86 ± 7.03	12.61 ± 6.02	ns
maxGH during sleep (ng/ml)	16.47 ± 9.31	13.89 ± 8.95	10.86 ± 5.52	ns
IGF-1 (ng/ml)	120.26 ± 65.57^{a,b}	192.30 ± 116.67^{a}	216.79 ± 120.01^{b}	<0.01
IGFBP-3 (µg/ml)	3.81 ± 1.09	4.47 ± 1.13	4.81 ± 1.84	ns
IGF-1/IGFBP-3 molar ratio	0.16 ± 0.06^{a}	0.23 ± 0.12^{a}	0.24 ± 0.11	<0.05
IGF-1 SDS	-1.22 ± 1.19	-1.10 ± 1.18	-0.53 ± 0.78	ns

^{a,b,c}values in the same row with different superscripts are significantly differed (p<0.05); BMI – body mass index, SDS – standard deviation score, TG - triglycerides, CH – cholesterol, IGF-1 – insulin-like growth factor 1, IGFBP-3 – insulin-like growth factor binding proteins 3, maxGH – maximal GH concentration during stimulation tests or during sleep.

concentration (**Table 2**). There was no significant correlations of the body mass index (i.e. BMI SDS for CA or for HA), leptin, adiponectin or resistin concentration with TSH and FT4 concentrations. However, we noticed significant positive correlations between leptin/adiponectin ratio and: cholesterol, LDL-fraction of cholesterol and triglicerides (**Table 2**).

DISCUSSION

In the group of children with ISS included in our study, no increase in TSH serum level was observed with increasing children's BMI and leptin concentration. In the subgroup of obese short children, TSH levels were not higher than in other subgroups. Although the group we studied was small (which is a limitation of our work), it seems that our results are worth showing. Many studies on both children and adults, conducted on large groups of patients, have shown that TSH levels correlate positively with BMI and leptin (28, 29). However, an interesting aspect that distinguishes our study is that it concerned children with short stature. Higher leptin concentrations may partially explain the effect of obesity on thyroid function, perhaps through the effect of leptin on TSH secretion, as this increase has been shown to be correlated with leptin regardless of BMI (28, 29). Thus, it is surprising that we did not find such a relationship in our group. Although the thesis concerning the increase in TSH in obesity due to the increased production of pro-TRH through the stimulation of the hypothalamus by leptin is plausible, there are certainly other mechanisms that influence (modulate) this relationship. One of them may be the excessive concentration of ghrelin, which we wrote about in the previous work (10). It is also possible that inflammation drives the changes in TSH and thyroid hormone levels in obesity. Weight reduction is likely to be associated with a reduction in inflammation and may explain

TABLE 2 | The correlation of TSH, FT4 and lipids concentration with BMISDS and selected adipocytokines.

	BMI SDS for HA	Leptin	Adiponectin	Leptin/adiponectin ratio	Resistin
TSH	0.06	-0.09	+0.04	-0.13	+0.07
FT4	-0.02	-0.02	+ 0.2*	-0.18	+0.1
Cholesterol	+0.21	+0.2	+0.03	+0.29*	-0.19
LDL-Cholesterol	+0.24*	+0.14	-0.03	+0.26*	-0.12
HDL-Cholesterol	+0.14*	+0.07	+0.21	0.05	-0.15
Triglicerides	-0.04	+0.26*	-0.05	+0.28*	+0.11

**p < 0.05; BMI SDS for HA - body mass index standard deviation score for height age*

It was observed that the higher the leptin the lower the GH secretion during the test with clonidine (r=-0.32, p < 0.05), as well as the negative correlation between leptin/adiponectin ratio and GH secretion during the test with clonidine (r=-0.39, p < 0.05) and during sleep (r=-0.2, p < 0.05).

the observed correlation between weight loss and a reduction in TSH (12). We have also recently analysed the prevalence of elevated TSH in children with acute respiratory infection and found elevated TSH in 10% of the cases, which returned to normal in all children shortly after recovery (30).

The slightly elevated TSH levels are seen in obese individuals, but not in all of them (12, 31–33). In a study by Habib et al. (5), TSH and FT4 levels were assessed in 850 children aged 2 to 18 years, and it was found that elevated TSH levels are observed in 17.2% of overweight and 20.5% of obese children; in contrast to 9.9% of slim and only 3.8% of normal weight children. In turn, in the study by Wolters et al. (12), elevated TSH was observed in 39% of 477 obese children, however the authors set the cut-off point for the elevated TSH concentration at a lower level, i.e. 3.0 mIU/l.

Thus, the prevalence of hyperthyrotropinemia among obese patients differs in individual analyses.

Meanwhile, in 2020, Wang et al. (11) found that increased TSH levels are more often observed in girls with generalized obesity compared to those with central obesity. We did not analyse the type of obesity in our research. It should also be taken into account that among our patients there were no patients with extreme obesity. The highest value of BMI was +3.9 SD.

In 2019, Ruszała et al. (14) assessed the influence of the thyroid axis dysfunction on the occurrence of metabolic obesity complications. They analysed a group of 100 obese children, where 25 children had features of the metabolic syndrome and 75 did not. The authors found no case of overt thyroid disease within the whole analyzed group. There were no significant differences in mean TSH, FT4, and FT3 levels in patients with and without the metabolic syndrome. Moreover, an elevated TSH level was found in 8% of obese patients with the metabolic syndrome and 24% of obese patients without it. The authors concluded that an isolated increased TSH level is not common in obese adolescents and there is no correlation between TSH, FT3, FT4 levels and BMI SDS value. Moreover, isolated increased TSH levels were not associated with the occurrence of the metabolic syndrome in obese adolescents (14).

In the present study, we also analysed lipids profile. We found a significantly higher concentration of total cholesterol and LDL-cholesterol in the subgroup of children with obesity. However, we did not find any significant correlation between lipids and each of the analysed hormones (i.e. TSH or FT4). In particular, a positive correlation between proatherogenic lipids (cholesterol, LDL-cholesterol and triglycerides) concentrations and leptin/adiponectin ratio was found. It may suggest that the metabolic disorders which we observed were the result of too weak stimulation of pro-TRH and TSH by leptin (e.g, in certain disorders at the hypothalamic level) and, in consequence, the relative hypothyroidism. It may also be a possible explanation of an additional observation we made, namely a negative correlation between leptin concentration (or leptin/adiponectin ratio) and GH secretion during the stimulation test with clonidine. This phenomenon, observed in obese children, can be explained - among others - by the blocking effect of lipid disorders on GHRH and GH secretion (34, 35). Therefore, it is possible that some obese children experience a weaker action exerted by TRH and GHRH jointed on the level of the pituitary gland.

In turn, Bossowski et al. (25) explored other aspects of these issues. They analysed leptin, adiponectin and resistin in children with untreated Graves' disease and hypothyroidism in Hashimoto's thyroiditis. The authors showed higher adiponectin and lower resistin levels in hyperthyroidism than in hypothyroidism. The analysis of leptin levels revealed no significant differences between children with subclinical hypothyroidism and untreated Graves' disease. Thus, their research also supports the idea that leptin and TSH levels are in a poor cause-and-effect relationship. However, they suggested that disturbances in thyroid hormones in thyroid diseases have a significant effect on the levels of adiponectin and resistin released by adipose tissue (25). We also observed the same relationship between FT4 and adiponectin concentrations in the analysed group of children. It is to be noted that a higher FT4 concentration should be beneficial for the decreased leptin/adiponectin ratio. However, in the analysed group of short children we did not find that relationship. Thus, the observed phenomenon of increased TSH in some obese children is probably multifactorial, and in children without thyroid disease could trigger protective effects, but it does not seem to apply to all obese children and especially to obese children with idiopathic short stature.

The lack of leptin influence on TSH concentration could indicate wide ranging disturbances of hypothalamic signals, and consequently be the cause of inappropriate GH secretion.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Bioethical Committee at the Polish Mother's Memorial Hospital-Research Institute (PMMH-RI) in Lodz. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

Conceptualization, KA and RS. Methodology, RS and KA. Resources, RS. Writing—original draft preparation, KA and ZA. Writing—review and editing, AL. Supervision, AL and RS. All authors have read and agreed to the published version of the manuscript.

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7. Opinie Komisji Bioetycznych

Łódź, 23 września 2009 r.

KOMISJA
ETYKI BADAŃ NAUKOWYCH
Instytutu
Centrum Zdrowia Matki Polki

Dr n. med. Renata Stawerska
Klinika Endokrynologii i Chorób Metabolicznych
Instytutu Centrum Zdrowia Matki Polki

Komisja Etyki Badań Naukowych Instytutu Centrum Zdrowia Matki Polki na posiedzeniu w dniu 23 września 2009 r. rozpatrywała wniosek dotyczący pracy:

„Wpływ wybranych czynników infekcyjnych przewodu pokarmowego na wydzielanie enterohormonów (greliny, insuliny, leptyny, neuropeptydu Y, peptydu YY, oreksyny i alfaMSH) u dzieci z niedoborem wrostu i masy ciała: jako zjawisko molekularnej mimikry"

Opinia

Komisja Etyki Badań Naukowych przy Instytucie Centrum Zdrowia Matki Polki zapoznała się z ww projektem eksperymentu medycznego, przeanalizowała wniosek, wysłuchała opinii recenzenta o przedstawionym projekcie i wyniku przeprowadzonej dyskusji oraz tajnego głosowania, po rozważeniu kryteriów etycznych oraz celowości i wykonalności projektu pozytywnie zaopiniowała projekt eksperymentu medycznego.

Przewodniczący:

Prof. dr hab. med. Andrzej Chilarski .....

Członkowie:

Prof. dr hab. med. Tadeusz Biegański .....

Ks. Mateusz Cieplucha .....

Dr n. med. Paweł Czekalski .....

Mgr Grażyna Korybut .....

Prof. dr hab. med. Izabela Płaneta-Małecka .....

Prof. dr hab. med. Krzysztof Szaflik .....

Dr hab. filozofii Wojciech Sztombka .....

Prof. dr n. med. Krzysztof Szyłło .....

Prof. dr hab. med. Jan Wilczyński .....

Prof. dr hab. med. Teresa Woźniakowska-Gęsicka .....

Komisja Bioetyczna
przy Instytucie Centrum Zdrowia Matki Polki
93-338 Łódź, Rzgowska 281/289 tel. (42) 271 11 28
e-mail biuro81@iczmp.edu.pl

Łódź, dnia 11 września 2018 r.

Dr hab. n. med. Renata Stawerska
Klinika Endokrynologii i Chorób Metabolicznych
Instytutu Centrum Zdrowia Matki Polki

Komisja Bioetyczna przy Instytucie Centrum Zdrowia Matki Polki działając zgodnie z zasadami Dobrej Praktyki Klinicznej na posiedzeniu w dniu 11 września 2018 r. rozpatrywała wniosek dotyczący pracy:

„Wpływ stanu zapalnego związanego z infekcją dróg oddechowych na stężenie TSH i FT_4 u dzieci zgłaszających się do Poradni Podstawowej Opieki Zdrowotnej."

Zespół badaczy:
1. Dr hab. n. med. Renata Stawerska
2. Lek. Katarzyna Adamczewska
3. Prof. dr hab. n. med. Andrzej Lewiński

Opinia Nr 69/2018

Komisja Bioetyczna przy Instytucie Centrum Zdrowia Matki Polki zapoznała się z ww projektem eksperymentu medycznego, przeanalizowała wniosek, wysłuchała opinii recenzenta o przedstawionym projekcie i wyniku przeprowadzonej dyskusji oraz tajnego głosowania, po rozważeniu kryteriów etycznych oraz celowości i wykonalności projektu pozytywnie zaopiniowała projekt eksperymentu medycznego.

Uchwałę podjęto jednogłośnie.
Uchwałę podjęto przy sprzeciwie .....

Przewodnicząca:

Dr hab. med. Iwona Maroszyńska, prof. ICZMP.....

Zastępca Przewodniczącej:

Prof. dr hab. n. farm. Daria Orszulak-Michalak .....

Członkowie:

Mec. Michał Araszkiewicz .....

Prof. dr hab. n. med. Tadeusz Biegański .....

Dr n. med. Paweł Czekalski .....

Dr hab. n. med. Piotr Grzelak, prof. ICZMP .....

Mgr Grażyna Korybut .....

Dr n. med. Michał Krekora .....

Prof. dr hab. med. Jacek Rysz .....

Dr n. filozofii Wojciech Sztombka .....

Ks. dr hab. Jan Wolski .....

Dr hab. n. med. Marek Zadrożny, prof. ICZMP .....

Prof. dr hab. n. med. Krzysztof Zeman .....

8. Oświadczenia współautorów publikacji

Informacja o charakterze udziału współautorów w publikacjach wraz z szacunkowym określeniem procentowego wkładu

Adamczewska K, Adamczewski Z, Stasiak M, Lewiński A, Stawerska R. Transient Hyperthyrotropinemia in Outpatient Children with Acute Infections of the Respiratory System. Int J Environ Res Public Health. 2021 Apr 13;18(8):4115.

Imię i nazwisko współautora	Charakter udziału	Procentowy wkład
Doktorant – lek. Katarzyna Adamczewska	Stworzenie koncepcji pracy, udział w planowaniu badania, rekrutowanie pacjentów, opracowanie i analiza wyników, przygotowanie manuskryptu	55%
dr hab. n. med. Zbigniew Adamczewski	Udział w przygotowaniu manuskryptu	10%
dr hab. n. med. Magdalena Stasiak	Udział w przygotowaniu ostatecznej wersji manuskryptu i recenzja pracy	5%
prof. dr hab. n. med. Andrzej Lewiński	Udział w przygotowaniu ostatecznej wersji manuskryptu, recenzja pracy i nadzór merytoryczny	10%
dr hab. n. med. Renata Stawerska	Stworzenie koncepcji pracy, udział w planowaniu badania, pomoc przy przygotowaniu manuskryptu i nadzór merytoryczny	20%

Oświadczam, że wszyscy współautorzy wyrazili zgodę na wykorzystanie powyższej publikacji w pracy doktorskiej lek. Katarzyny Adamczewskiej

Podpis doktorantki

Katarzyna Adamczewska

Łódź, dnia 7.03.2022r.

lek. Katarzyna Adamczewska
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Transient Hyperthyrotropinemia in Outpatient Children with Acute Infections of the Respiratory System", oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to: stworzenie koncepcji pracy, udział w planowaniu badania, rekrutowanie pacjentów, opracowanie i analiza wyników, przygotowanie manuskryptu. Mój udział procentowy w przygotowaniu powyższej publikacji szacuję na około 55%.

Katarzyna Adamczewska
.....

(podpis współautora)

Łódź, dnia 7.03.2022r.

dr hab. n. med. Zbigniew Adamczewski
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Transient Hyperthyrotropinemia in Outpatient Children with Acute Infections of the Respiratory System", oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to: udział w przygotowaniu manuskryptu.
Mój udział procentowy w przygotowaniu powyższej publikacji szacuję na około 10%.

Jednocześnie wyrażam zgodę na uznanie, iż w/w praca przedłożona przez lek. Katarzynę Adamczewską, jako część cyklu publikacyjnego będącego podstawą ubiegania się o nadanie stopnia doktora nauk medycznych, stanowi jej indywidualny wkład w rozwój medycyny.

.....

(podpis współautora)

Łódź, dnia 7.03.2022r.

dr hab. n. med. Magdalena Stasiak
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Transient Hyperthyrotropinemia in Outpatient Children with Acute Infections of the Respiratory System", oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to: udział w przygotowaniu ostatecznej wersji manuskryptu i recenzja pracy.

Mój udział procentowy w przygotowaniu powyższej publikacji szacuję na około 5%.

Jednocześnie wyrażam zgodę na uznanie, iż w/w praca przedłożona przez lek. Katarzynę Adamczewską jako część cyklu publikacyjnego będącego podstawą ubiegania się o nadanie stopnia doktora nauk medycznych, stanowi jej indywidualny wkład w rozwój medycyny.

.....

(podpis współautora)

Łódź, dnia 7.03.2022r.

prof. dr hab. n. med. Andrzej Lewiński
.....
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „ Transient Hyperthyrotropinemia in Outpatient Children with Acute Infections of the Respiratory System",
oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:
udział w przygotowaniu ostatecznej wersji manuskryptu, recenzja pracy i nadzór merytoryczny.
Mój udział procentowy w przygotowaniu powyższej publikacji szacuję na około 10%.

Jednocześnie wyrażam zgodę na uznanie, iż w/w praca przedłożona przez lek. Katarzynę Adamczewską, jako część cyklu publikacyjnego będącego podstawą ubiegania się o nadanie stopnia doktora nauk medycznych, stanowi jej indywidualny wkład w rozwój medycyny.

.....
(podpis współautora)

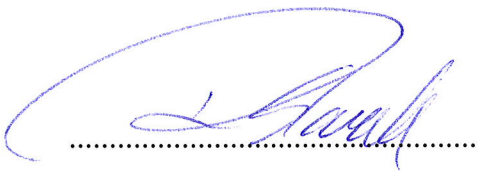
Łódź, dnia 7.03.2022r.

dr hab. n. med. Renata Stawerska
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Transient Hyperthyrotropinemia in Outpatient Children with Acute Infections of the Respiratory System", oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:
stworzenie koncepcji pracy, udział w planowaniu badania i przygotowaniu manuskryptu oraz nadzór merytoryczny.
Mój udział procentowy w przygotowaniu powyższej publikacji szacuję na około 20%.

Jednocześnie wyrażam zgodę na uznanie, iż w/w praca przedłożona przez lek. Katarzynę Adamczewską, jako część cyklu publikacyjnego będącego podstawą ubiegania się o nadanie stopnia doktora nauk medycznych, stanowi jej indywidualny wkład w rozwój medycyny.



.....

(podpis współautora)

Adamczewska K, Adamczewski Z, Łupińska A, Lewiński A, Stawerska R. Strong Positive Correlation between TSH and Ghrelin in Euthyroid Non-Growth Hormone-Deficient Children with Short Stature. Molecules. 2020 Aug 27;25(17):3912.

Imię i nazwisko współautora	Charakter udziału	Procentowy wkład
Doktorant – lek. Katarzyna Adamczewska	Stworzenie koncepcji pracy, udział w planowaniu badania, opracowanie i analiza wyników, przygotowanie manuskryptu	50%
dr hab. n. med. Zbigniew Adamczewski	Udział w przygotowaniu manuskryptu	10%
dr n. med. Anna Łupińska	Rekrutowanie pacjentów	5%
prof. dr hab. n. med. Andrzej Lewiński	Udział w przygotowaniu ostatecznej wersji manuskryptu, recenzja pracy i nadzór merytoryczny	10%
dr hab. n. med. Renata Stawerska	Stworzenie koncepcji pracy, udział w planowaniu badania, rekrutowanie pacjentów i nadzór merytoryczny	25%

Oświadczam, że wszyscy współautorzy wyrazili zgodę na wykorzystanie powyższej publikacji w pracy doktorskiej lek. Katarzyny Adamczewskiej

Podpis doktorantki

Katarzyna Adamczewska

Łódź, dnia 7.03.2022r.

lek. Katarzyna Adamczewska
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Strong Positive Correlation between TSH and Ghrelin in Euthyroid Non-Growth Hormone-Deficient Children with Short Stature", oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to: stworzenie koncepcji pracy, udział w planowaniu badania, opracowanie i analiza wyników, przygotowanie manuskryptu. Mój udział procentowy w przygotowaniu powyższej publikacji szacuję na około 50%.

Katarzyna Adamczewska
.....

(podpis współautora)

Łódź, dnia 7.03.2022r.

dr hab. n. med. Zbigniew Adamczewski
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Strong Positive Correlation between TSH and Ghrelin in Euthyroid Non-Growth Hormone-Deficient Children with Short Stature", oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to: udział w przygotowaniu manuskryptu.
Mój udział procentowy w przygotowaniu powyższej publikacji szacuję na około 10%.

Jednocześnie wyrażam zgodę na uznanie, iż w/w praca przedłożona przez lek. Katarzynę Adamczewską, jako część cyklu publikacyjnego będącego podstawą ubiegania się o nadanie stopnia doktora nauk medycznych, stanowi jej indywidualny wkład w rozwój medycyny.

.....

(podpis współautora)

Łódź, dnia 7.03.2022r.

dr n. med. Anna Łupińska
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Strong Positive Correlation between TSH and Ghrelin in Euthyroid Non-Growth Hormone-Deficient Children with Short Stature", oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to: udział w rekrutowaniu pacjentów.
Mój udział procentowy w przygotowaniu powyższej publikacji szacuję na około 5%.

Jednocześnie wyrażam zgodę na uznanie, iż w/w praca przedłożona przez lek. Katarzynę Adamczewską, jako część cyklu publikacyjnego będącego podstawą ubiegania się o nadanie stopnia doktora nauk medycznych, stanowi jej indywidualny wkład w rozwój medycyny.

Anna Łupińska
.....
(podpis współautora)

Łódź, dnia 7.03.2022r.

prof. dr hab. n. med. Andrzej Lewiński
.....
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „ Strong Positive Correlation between TSH and Ghrelin in Euthyroid Non-Growth Hormone-Deficient Children with Short Stature”, oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to: udział w przygotowaniu ostatecznej wersji manuskryptu, recenzja pracy i nadzór merytoryczny.
Mój udział procentowy w przygotowaniu powyższej publikacji szacuję na około 10%.

Jednocześnie wyrażam zgodę na uznanie, iż w/w praca przedłożona przez lek. Katarzynę Adamczewską, jako część cyklu publikacyjnego będącego podstawą ubiegania się o nadanie stopnia doktora nauk medycznych, stanowi jej indywidualny wkład w rozwój medycyny.

.....
(podpis współautora)

Łódź, dnia 7.03.2022r.

dr hab. n. med. Renata Stawerska
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Strong Positive Correlation between TSH and Ghrelin in Euthyroid Non-Growth Hormone-Deficient Children with Short Stature", oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:
stworzenie koncepcji pracy, udział w planowaniu badania, rekrutowanie pacjentów i nadzór merytoryczny.
Mój udział procentowy w przygotowaniu powyższej publikacji szacuję na około 25%.

Jednocześnie wyrażam zgodę na uznanie, iż w/w praca przedłożona przez lek. Katarzynę Adamczewską, jako część cyklu publikacyjnego będącego podstawą ubiegania się o nadanie stopnia doktora nauk medycznych, stanowi jej indywidualny wkład w rozwój medycyny.

.....

(podpis współautora)

Adamczewska K, Adamczewski Z, Lewiński A, Stawerska R. Leptin does not stimulate TSH secretion in obese short children. Front. Endocrinol. 2022; 13:838881.

Imię i nazwisko współautora	Charakter udziału	Procentowy wkład
Doktorant – lek. Katarzyna Adamczewska	Stworzenie koncepcji pracy, udział w planowaniu badania, opracowanie i analiza wyników, przygotowanie manuskryptu	50%
dr hab. n. med. Zbigniew Adamczewski	Pomoc przy przygotowaniu manuskryptu	10%
prof. dr hab. n. med. Andrzej Lewiński	Pomoc przy przygotowaniu ostatecznej wersji manuskryptu, recenzja pracy i nadzór merytoryczny	10%
dr hab. n. med. Renata Stawerska	Stworzenie koncepcji pracy, udział w planowaniu badania, rekrutowanie pacjentów i nadzór merytoryczny	30%

Oświadczam, że wszyscy współautorzy wyrazili zgodę na wykorzystanie powyższej publikacji w pracy doktorskiej lek. Katarzyny Adamczewskiej

Podpis doktorantki

Katarzyna Adamczewska

Łódź, dnia 7.03.2022r.

lek. Katarzyna Adamczewska
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Leptin does not stimulate TSH secretion in obese short children", oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to: stworzenie koncepcji pracy, udział w planowaniu badania, opracowanie i analiza wyników, przygotowanie manuskryptu.
Mój udział procentowy w przygotowaniu powyższej publikacji szacuję na około 50%.

Katarzyna Adamczewska
.....
(podpis współautora)

Łódź, dnia 7.03.2022r.

dr hab. n. med. Zbigniew Adamczewski
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Leptin does not stimulate TSH secretion in obese short children", oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to: udział w przygotowaniu manuskryptu.
Mój udział procentowy w przygotowaniu powyższej publikacji szacuję na około 10%.

Jednocześnie wyrażam zgodę na uznanie, iż w/w praca przedłożona przez lek. Katarzynę Adamczewską, jako część cyklu publikacyjnego będącego podstawą ubiegania się o nadanie stopnia doktora nauk medycznych, stanowi jej indywidualny wkład w rozwój medycyny.

.....

(podpis współautora)

Łódź, dnia 7.03.2022r.

prof. dr hab. n. med. Andrzej Lewiński
.....
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Leptin does not stimulate TSH secretion in obese short children", oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to: udział w przygotowaniu ostatecznej wersji manuskryptu, recenzja pracy i nadzór merytoryczny.

Mój udział procentowy w przygotowaniu powyższej publikacji szacuję na około 10%.

Jednocześnie wyrażam zgodę na uznanie, iż w/w praca przedłożona przez lek. Katarzynę Adamczewską, jako część cyklu publikacyjnego będącego podstawą ubiegania się o nadanie stopnia doktora nauk medycznych, stanowi jej indywidualny wkład w rozwój medycyny.

.....
(podpis współautora)

Łódź, dnia 7.03.2022r.

dr hab. n. med. Renata Stawerska
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Leptin does not stimulate TSH secretion in obese short children", oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to: stworzenie koncepcji pracy, udział w planowaniu badania, rekrutowanie pacjentów i nadzór merytoryczny.

Mój udział procentowy w przygotowaniu powyższej publikacji szacuję na około 30%.

Jednocześnie wyrażam zgodę na uznanie, iż w/w praca przedłożona przez lek. Katarzynę Adamczewską, jako część cyklu publikacyjnego będącego podstawą ubiegania się o nadanie stopnia doktora nauk medycznych, stanowi jej indywidualny wkład w rozwój medycyny.

.....

(podpis współautora)