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The between-day reproducibility of fasting, satiety-related analytes, in 8 to 11 year-old boys

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HIGHLIGHTS

- Between-day reproducibility of glucose and plasma GLP-$1_{7–36}$ by fingertip capillary sampling displayed low typical and random imprecision in the 21, 8–11 y boys.
- When analysed according to body composition, there was good reproducibility for glucose in the overweight/obese boys and for plasma GLP-$1_{7–36}$, in those with lean body mass.
- Comparison of blood glucose obtained by fingertip capillary sampling is possible between lean and overweight/obese 8–11 y boys.
- Comparison of fasting plasma GLP-$1_{7–36}$ according to body weight is inappropriate due to high imprecision observed in lean boys, between-days.
- The measurement of satiety-related analytes by fingertip capillary sampling could provide better understanding of mechanisms that affect appetite and feeding behaviour in children.

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ABSTRACT

The aim of the present study was to establish the between-day reproducibility of fasting plasma GLP-1_{7-36}, glucagon, leptin, insulin and glucose, in lean and overweight/obese 8–11 year-old boys. A within-group study design was utilised wherein the boys attended two study days, separated by 1 week, where a fasting fingertip capillary blood sample was obtained. Deming regression, mean difference, Bland-Altman limits of agreement (LOA) and typical imprecision as a percentage coefficient of variation (CV %), were utilised to assess reproducibility between-days. On a group level, Deming regression detected no evidence of systematic or proportional bias between-days for all of the satiety-related analytes however, only glucose and plasma GLP-1_{7-36} displayed low typical and random imprecision. When analysed according to body composition, good reproducibility was maintained for glucose in the overweight/obese boys and for plasma GLP-1_{7-36}, in those with lean body mass. The present findings demonstrate that the measurement of glucose and plasma GLP-1_{7-36} by fingertip capillary sampling on a group level, is reproducible between-days, in 8–11 year-old boys. Comparison of blood glucose obtained by fingertip capillary sampling can be made between lean and overweight/obese 8–11 year-old boys. Presently, the comparison of fasting plasma GLP-1_{7-36} according to body weight is inappropriate due to high imprecision observed in lean boys between-days. The use of fingertip capillary sampling in the measurement of satiety-related analytes has the potential to provide a better understanding of mechanisms that affect appetite and feeding behaviour in children.

1. Introduction

Human appetite and the regulation of feeding behaviour are sophisticated processes. Emerging evidence confirms the control of appetite and regulation of feeding behaviour is primarily governed through interaction between the nervous and digestive systems, via the enteric nervous system (ENS) [1]. There are numerous analytes which elicit episodic (short-term) and tonic (long-term) properties, relaying information through the gut-brain axis to regulate satiety. The present work will focus on glucagon-like-peptide 1 (GLP-1_{7-36}), glucagon, glucose (episodic analytes), insulin and leptin (tonic analytes). The aforementioned analytes represent several commonly measured metabolic variables documented as having fundamental roles in satiety signalling [2] and thus contribute to human energy balance [3–6].

During consumption of a meal, GLP-1_{7-36} is released by the endocrine L-cells as nutrients are detected in the duodenum.

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Appearance of GLP-$1_{7\text{-}36}$ in the circulation is bi-phasic, occurring within 10 to 15 min [7,8] and at 30 to 60 min [9] following ingestion. The effects of GLP-$1_{7\text{-}36}$ via the ENS are to inhibit gastric emptying and intestinal motility, a process termed the 'ileal brake' which brings about meal termination [6]. Levels of circulating GLP-$1_{7\text{-}36}$ can be elevated for more than 3 h following a meal [10]. It has been suggested that GLP-$1_{7\text{-}36}$ not only acts to restrict food intake but also functions to extend the time before any further eating episode can occur [10]. Recent evidence indicates that glucagon is able to signal the brain through vagal afferent neurons, to effect meal termination and may also decrease meal size [11]. Primarily glucagon opposes the actions of insulin [11,12] which is released due to the detection of glucose in the blood. The main function of insulin in satiety therefore, is to enable uptake of glucose and reduce levels of the blood sugar [13] in accordance with the 'glucostatic theory' [14]. The short-term actions of GLP-$1_{7\text{-}36}$, insulin and glucagon, are in contrast to leptin which has long term anorectic properties. Leptin is an adipokine largely produced by adipocytes and is correlated with white adipose tissue [15]. When an individual is in a positive energy balance state, circulating plasma leptin is increased which facilitates a reduction in food intake, until energy balance is restored [12]. Leptin also has specific short term functions that bring about a reduction in meal size. It appears to do this by acting on taste sensitivity through the hyperpolarization of taste buds on the tongue [16] which reduces the positive reinforcing effects of food ingestion on the brain [17].

In England, 19.1% of children are currently obese [18] and it appears to have greater prevalence in boys during mid-to-late childhood (8–11 years) [19]. Assessment of the aforementioned analytes in paediatric populations could provide essential information in relation to the regulation of appetite and feeding behaviour in children. To the author's knowledge, appetite research that quantifies glucose, GLP-$1_{7\text{-}36}$, insulin, glucagon and leptin in healthy paediatric populations, particularly 8–11 year-old boys is sparse and is likely due to the sampling methods invariably utilised.

Generally, in research and clinical practice, blood is obtained by antecubital-venous or arterio-venous sampling. For research with vulnerable populations such as children, antecubital-venous sampling is invasive and may even be deemed as unethical. Recent research from our laboratory has examined the agreement and reproducibility of plasma GLP-$1_{7\text{-}36}$, glucagon, leptin and insulin, between fingertip capillary blood and antecubital-venous sampling in healthy adults [20]. Green and colleagues (2014) [20] demonstrated that fingertip capillary blood sampling provided a comparable and reproducible alternative to antecubital-venous, to quantify glucagon and to lesser degree, GLP-$1_{7\text{-}36}$, leptin and insulin. Such a method is far less invasive than venous sampling, and thus represents a more suitable procedure for use in paediatric populations [20].

To the best of our knowledge, evidence of between-day reproducibility in fasted plasma GLP-$1_{7\text{-}36}$, glucagon, leptin and insulin exist only for healthy adults, for traditional methods of blood sampling [10,21,22] and fingertip capillary sampling [20]. Currently, there is no understanding of between-day reproducibility of fasted plasma GLP-$1_{7\text{-}36}$, glucagon, leptin and insulin obtained from fingertip capillary blood in children. In view of the less invasive nature of fingertip capillary sampling, prior to short-term intervention in appetite-related studies with children, it seems prudent to establish between-day reproducibility in fasted levels of these analytes of interest. Knowledge of the between-day reproducibility will inform researchers whether changes are due to intervention and not imprecision related to sample handling, analytical procedures and equipment, or disparity in biological responses. Consequently, the aim of the present study is to establish the between-day reproducibility of fasting plasma GLP-$1_{7\text{-}36}$, glucagon, insulin, leptin and blood glucose obtained by fingertip capillary sampling, in 8–11 year-old lean and overweight/obese boys.

2. Methods

2.1. Study design

A within-group study design was utilised to establish between-day reproducibility in fasting plasma GLP-$1_{7\text{-}36}$, glucagon, insulin and leptin and blood glucose obtained from fingertip capillary blood, in 8–11 year-old boys.

The study was conducted according to 2013 Declaration of Helsinki (World Medical Association. 2013) and was approved by the University of Northumbria, Faculty of Health and Life Sciences Ethics Committee. Written informed consent was obtained from each child's parent or main carer and assent was given by the child prior to data collection.

2.2. Participants

Boys aged 8–11 years were recruited from a primary school located within the city of Newcastle upon Tyne (North East England, UK). To enable recruitment, consent was obtained from the Head Teacher of the school they attended. A recruitment pack was distributed to all eligible boys who expressed an interest in participating and they were asked to take this home to their parent (or main carer). The pack contained a letter addressed to their parent/main carer with a full explanation of the study and consent forms for them and their child (if able) to sign and return to school. Signed consent was received from 24 boys, of which 23 participated in the study. Boys were excluded from participating if they were diabetic or took any form of medication known to affect taste, smell or appetite.

2.3. Study protocol

Prior to the first visit to the University laboratory, each boy was provided with a food diary. With the help of their parent (or main carer) they were requested to weigh and record all foods and fluids consumed from 1700 h the day before each visit until 2100 h, at which point they were required to begin a 12 h overnight fast. Following the first visit, they were provided with a copy of the food diary so that their food and fluid intake could be replicated prior to visit two. With the assistance of the parent (or main carer) they were asked to refrain from sport or physical activity from 1700 h until arrival at school on the morning of each visit.

The boys were required to attend the University laboratory on two different days, separated by 1 week. On the morning of each visit, following a 12 h overnight fast, the boys attended school at 0830 h. From waking, they were permitted to drink only water and with the assistance of their parent (or main carer) were asked to note this amount in the food diary to enable replication prior to the second visit. For logistical reasons, the boys were organised into testing groups of five to seven. At school (0830 h), the boys were met by two members of the research team and transported to the University for 0845 h so that they could each provide one fasted capillary blood sample.

During the first visit, the stature and seated height of each boy were measured to the nearest 0.01 m using a Harpenden Portable Stadiometer (Holtain Limited, Pembs, UK) to calculate age (year) from peak height velocity (APHV) [23]. Body mass was measured to the nearest 0.1 kg using portable SECA scales (SECA United Kingdom) whilst wearing light clothing. Waist circumference was measured to the nearest 0.01 m with a non-elastic flexible tape at each boy's natural waist whilst standing, as a measure of central adiposity [24]. In both visits, immediately following the collection of the blood sample, each boy was provided with breakfast, after which they were escorted back to school by two researchers.